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THE RENAL ARTERIOLAR CHANGES IN THE ANURIC CRUSH SYNDROME*

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The most striking changes reported hitherto in the kidneys of patients with the anuric crush syndrome concern the ascending limb of the loop of Henle and the second convoluted tubule,[1,2] and have been extensively discussed by Bywaters and Dible.[2] They account for the loss of selective reabsorption observed in cases of fatal crush syndrome but they fail to give a satisfactory explanation of the marked oliguria and the gradual increase of blood pressure.

In a recent paper[3] I have drawn attention to the renal *arteriolar* changes in crush syndrome. Their elucidation requires familiarity with the existence of a juxta-glomerular apparatus and, possibly, acceptance of glandular function for certain cells in the media of the renal arterioles.[4-8]

The purpose of this paper is to analyze these changes in detail and to attempt a precise definition of the renal lesion which may follow the release of toxic substances by crushing injuries to the muscles of the limbs.

MATERIALS AND METHOD

I have investigated the histologic changes in four kidneys from patients with crush wounds (cases A-1, A-3, A-4, A-5). The kidneys were swollen, edematous, and grayish pink. They were fixed in a 10 per cent solution of neutral formalin and had been submitted to me by pathologists of the Royal Canadian Army Medical Corps stationed in Belgium. The victims were young enlisted men in the Canadian Army who had died 4 to 9 days after crushing injuries and after the development of marked oliguria and even anuria (case A-4). Unfortunately, no detailed clinical or post-mortem report was available. However, in cases A-3 and A-5 the blood pressure had been measured once on the fourth day: in the former the systolic blood pressure was 150 mm. of

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Hg and the diastolic was 70 mm. of Hg; in the latter the systolic blood pressure was 155 mm. of Hg and the diastolic was 75 mm. of Hg.

Sections from paraffin blocks were stained by Masson's trichrome iron-hematoxylin, fuchsin, and aniline blue.

A case of "traumatic uremia" observed by me during the war of 1914–18 is also included. The clinical data in this instance are summarized as follows: V. d. B., 23 years old; wounded in the shoulder by a shell splinter; left axillary artery torn with formation of a large hematoma, accompanied by hemorrhagic shock. Ligature of the left axillary artery and vein was performed and a blood transfusion was given. Incipient ischemic gangrene of the left arm appeared on the fifth day. Oliguria became notable (urinary output: maximum, 285 cc.; none on the sixth day). Urine: clear, albumin in small amount; uremic symptoms included vomiting. Blood pressure: 60/50 mm. of Hg on admission; 130/70 on the second and third days; 145/70 on the fourth; 140/60 on the fifth; 170/60 on the sixth day. Hemodilution was shown in the erythrocyte counts, which fell from 3,900,000, 5 hours after trauma, to 2,100,000 red blood cells per mm. shortly before death in uremic coma on the sixth day. Kidneys were fixed in Bouin's and Flemming's fluids. This case is designated as no. 76.

Microscopic sections in a sixth case were kindly put at my disposal (A-12-B) by Major McManus, Royal Canadian Army Medical Corps. These were from a case of traumatic uremia in a soldier, 40 years of age, following hemorrhage, extensive infection of muscle wounds, and blood transfusion.

Observations

The Glomeruli

As in previous papers, I have adopted Zimmermann's description of the structure of the glomerular tuft, according to which the loops of the latter are supported by a mesangium.[9] This formation consists of thin, often bifurcated lamellae, the ground substance of which comes out clearly with aniline blue (Fig. 1). The mesangium cells contain a round or oblong nucleus with densely packed chromatin. Their cytoplasm is scarce but shows slender expansions (at a magnification of 700 ×) which, stained with acid fuchsin, contrast sharply with the blue ground substance and give to the mesangium a heterogeneous aspect. Some of these expansions are in the free wall of the loops which constitutes the filtration membrane. This membrane is, in fact, formed by the coalescence of two basement membranes: that of the epithelial cells (Deckzellen) and that of the endothelial lining of the loops (Zimmermann [9]).

In ases of crush wound or traumatic uremia, the size of the glomerular tufts and the diameter of their loops varied. In some specimens the tufts were retracted (cases A-3 and A-4); in others, usually relaxed (cases A-1 and A-5). These variations did not depend on congestion or depletion of the loops. Most of the tufts were almost bloodless but they contained remnants of shrunken, hemolyzed erythrocytes (Figs. 1 and 2) which contrasted with the few preserved erythrocytes (stained black with iron hematoxylin) occasionally present in the loops and with the abundant erythrocytes observed in the intertubular capillaries. Other changes were noticed in the tuft. Often, the endothelial cells of the loops enlarged and resembled endothelial leukocytes (Fig. 1). Polymorphonuclear leukocytes were retained. Clusters of thrombocytes were found occasionally; hyaline thrombi, exceptionally (cases A-1 and A-4). When the tufts were relaxed (maximal relaxation found in case A-1) the loops were filled with vacuolated blood-plasma, faintly stained with aniline blue. In these cases, also, the infundibulum, from which originate the loops of the tuft (schema of Vimtrup), was large and distended with blood-plasma. Groups of normally stained erythrocytes were sometimes found to be floating in this fluid (case A-1). The wall of the infundibulum sometimes showed an increased number of spindle-shaped cells separated by fine collagen fibrils.

I have not noticed any striking changes in the mesangium except in cases A-1 and A-5 (Fig. 1). In many glomeruli of these cases the heterogenous structure of the mesangium had vanished and the ground substance had increased. Also pyknosis of the nuclei occurred. In apparently unchanged mesangia (cases A-3, A-4, 76, and A-12-B) hypertrophic or lobulated nuclei were discovered after an exhaustive examination.

The epithelial lining of the tuft offered no characteristic changes except in case A-1 where it was atrophic and degenerated in places (Fig. 1). The epithelial layer of Bowman's capsule rarely reacted and, if so, not very markedly. Apparently this occurred only when fibrinoid material, stained bright red with acid fuchsin, was deposited in the glomerular space—a very rare occurrence.

The distention of the glomerular capsule was caused either by the accumulation in the glomerular space of albuminous, grossly globoid (after fixation) material, or by the enlargement of the tuft itself (case A-1). It entailed the incorporation of the hypertrophic epithelial lining of the neck of the proximal convoluted tubule in the distended glomerular capsule.

When the tufts were excessively distended and when the mesangium was in regression, the so-called catarrh of the proximal convoluted

tubule was striking (case A-1). In its lumen the same kind of globoid material was found as in the glomeruli. On the other hand, this material was scanty and granular when the tuft was retracted and the mesangium was intact.

Hyalinized glomeruli were not more numerous than in normal persons of the same age.

Fat embolism of the tufts of moderate degree was an incidental finding.

The Post-Glomerular Arterioles

As a rule the lumina of the post-glomerular arterioles were wide and empty. Occasionally they contained a few hemolyzed erythrocytes close to the glomeruli and, exceptionally, normally stained erythrocytes. The media was unchanged and contained a small number of nongranular afibrillar cells. The presence of minute patches of subendothelial exudate was a constant feature (cases A-3, A-4, A-5, 76, and A-12-B, and especially A-1 and A-5). The lymphatics which run along the efferent arterioles were filled with mononuclear cells.

The Pre-Glomerular Arterioles

The pre-glomerular arterioles (juxta-glomerular apparatus included) show the most conspicuous changes. These come under two headings: (a) presence of numerous granular afibrillar cells in the media (Fig. 4), and (b) the presence of subendothelial patches of bluish stained transudate.

Before describing the granular afibrillar change of the media it must be emphasized that I have never found granular afibrillar cells in the arterioles of human kidneys unless there was hypertensive disease or a condition of anemia or serious liver damage. Graef [10] also found that in man the afibrillar cells of the renal arterioles normally are devoid of granules.

In crush wounds or traumatic uremia the intensity of the granular change of the smooth muscle cells varies. In case A-4 this transformation was confined chiefly to the juxta-glomerular apparatus (Fig. 6). In case 76 the muscular cells of the arterioles connected with superficial glomeruli had also become granular (Fig. 4). In case A-1, in which there was evidence of incipient damage to the tuft, the granular afibrillar transformation was maximal; it involved the afferent arteriole along its entire course and even the interlobular pre-arteriole. This granular reaction varied also qualitatively. In case A-4, in which the tufts were intact and retracted, the granules were very small and densely packed as in the kidney of the rabbit. In all other specimens they were coarse (Figs. 5 and 6) and sometimes irregular. The various types of granules

are demonstrated in Figure 1. Some stained bright red with acid fuchsin; others were black with iron hematoxylin. The fixation and staining technics may, to a certain extent, account for these variable staining properties; for the present it is advisable not to lay too much stress upon them. It must be emphasized, however, that the structure of the granular afibrillar cells varied considerably (Fig. 4). It was easy to follow the various stages of glandular activity which led ultimately to the dissolution of the granules and intense vacuolation. The latter process was very marked in cases A-1 and A-5 (Fig. 3): in many juxta-glomerular apparatuses the vacuolated cells predominated while the granular cells became rare. In the former, and to a lesser extent in case A-5, groups of granular smooth muscle cells had degenerated and shrunk. There were extracellular and intracellular lipoid drops in the juxta-glomerular apparatus of case 76 (Flemming's fixation). In cases A-3, A-4, A-12-B, and 76 the fibrillar smooth muscle cells formed the bulk of the media (Fig. 4); their myofibrils were conspicuous. They lay close to the endothelium while the afibrillar cells were in a marginal position. In the juxta-glomerular apparatus, however, the latter came close to the endothelium (Fig. 5). In case A-1 and sometimes in case A-5 most of the media cells were afibrillar; the lumen of the vas afferens was large and filled with pale-staining plasma. Some preparations from case A-1 suggested that the distention of the lumen results from a circulatory disturbance in the glomerular tuft.

In the pre-glomerular arterioles there were patches of subendothelial, finely vacuolated transudate; they stained blue with the Masson stain and must be considered as incipient arteriolosclerosis (Figs. 2 and 3). In cases A-1, A-4, 76, and A-12-B they were small and were found in the juxta-glomerular apparatus. In cases A-2 and A-5 they involved large segments of arterioles. Granular afibrillar cells were always found close to or in the patches. Often these cells are signs of incipient regression or excessive vacuolation (Figs. 2 and 3).

The Intertubular Capillaries

In contrast to the glomeruli, the intertubular capillaries were filled with intact erythrocytes. However, in some limited areas and especially around the glomeruli (cases A-3, A-4, A-12-B, and 76) a few capillaries were relaxed and devoid of erythrocytes. They contained coagulated plasma and occasionally monocytes, polymorphonuclear leukocytes, and blood-platelets in variable amount. In cases A-1 and A-5 these areas were larger; the basement membrane of the capillaries was thickened and collagenous fibrils were deposited between them, apparently without histiocytes (Fig. 7).

Except in the superficial layer of the cortex, interstitial edema was considerable and in some places was accompanied by histiocytic and fibroblastic proliferation. The latter was conspicuous in the boundary zone where the accumulation of pigment casts, the tubular damage, and the extrusion of casts were most marked (Bywaters and Dible [2]).

Interlobular Veins

The lesions of the veins which have been reported briefly in a previous paper [3] deserve special study, the result of which I intend to publish later.

Discussion

The changes described in this paper appear to be related to conditions prevailing shortly before death. The extreme glomerular ischemia, observed in my preparations, cannot have been of long duration because of the very slight morphologic damage to the tuft in all cases except two (cases A-1 and A-5). However, the retention of shrunken remnants of erythrocytes, the occasional presence of a few intact erythrocytes, the accumulation of polymorphonuclear leukocytes and blood-platelets, and, finally, the presence of blood-plasma in the loops of the tuft, suggest a sluggish or intermittently interrupted glomerular circulation for a considerable time before death. In most cases, glomerular filtration probably continued at a reduced rate but the filtrate contained an albuminous coagulum.

In discussing the mechanism which induces glomerular ischemia it must be kept in mind that the arterial blood pressure in patients suffering from crush wounds is either normal or high shortly before death; [11] that the lumen of the pre-glomerular arterioles is distended; that the number of the contractile elements of the arteriolar media has decreased; and that the retraction or relaxation of the tuft does not depend on the degree of vascularization of the tuft. In accordance with an opinion previously expressed,[7] I admit that the tone of the glomerular loops is controlled to some extent by the cells of the mesangium of Zimmermann which are of the same lineage as the muscle cells of the arterioles (de Winiwarter [12]) and are intact in most of my cases. The tuft is not a system of capillaries but an arteriolar segment adapted to the function of filtration.[7,8] I suggest that during the last days of the anuric crush syndrome the glomerular tuft contracts and in some cases ultimately relaxes. The relaxation should occur when the mesangium becomes functionally defective.

I also suggest that the numerical increase of the afibrillar granular cells in the media of the pre-glomerular arterioles is the result of a circulatory disturbance in the tufts, or in the arteriolocapillary junc-

tions further down stream, because a similar retrograde change occurs in the pre-glomerular arterioles of excessively ischemic kidneys. In the latter the cessation of glomerular circulation obviously precedes the obliteration of the afferent arterioles; these remain patent for some time and become blind alleys when the tufts are reduced to hyaline blocks; as the supply of oxygen to the muscular coating of these *culs-de-sac* decreases, there follows the appearance of granular cells all along their course.[7] According to my previous work,[7] confirmed by Dunihue and Candon,[13] these cells are believed to produce vasopressive or pre-vasopressive substance. There are reasons to believe that, normally, this substance acts on the neighboring contractile smooth muscle cells but overflows in the general circulation and causes hypertension when produced in excessive amount. I submit that the intensive glandular transformation of the renal arteriolar wall is one of the main factors responsible for the rise of blood pressure in crush syndrome. This claim is supported by the fact that whenever the afibrillar cells increase considerably in number and give evidence of enhanced glandular activity the renin content of the blood is also increased (abrupt rise of blood pressure after placing the Goldblatt clamp, hemorrhagic shock, eclampsia, fulminating acute glomerulonephritis (cf. Dexter *et al.*[14,15]).

This glandular transformation of the media of the pre-glomerular arterioles could be considered as morphologic evidence of a vasopressive compensatory reaction against a circulatory disturbance further down stream. Its intensity seems to be roughly proportional to the degree of the disturbance. In the Goldblatt experiment as long as the urinary function remains unimpaired the reaction is confined to the juxtaglomerular apparatus; but when, as the result of glomerular and tubular regression, the urinary function is reduced or lost, it extends up stream and involves even the interlobular pre-glomerular arterioles. A similar gradation is seen in the different specimens of crush-wound kidneys examined by me. The most extensive arteriolar changes occur when there is a marked deterioration of the intertubular and glomerular circulation. It is evident that this compensatory mechanism is efficient only when the peripheral circulation is slightly interfered with. In more severe cases no contractile substratum remains for the vasopressive substance to act upon because all of the fibrillar cells have become afibrillar and glandular.

I suggest that the correction of the deficiency of the arteriolo-capillary junctions and capillaries (excessive and prolonged vasoconstriction or excessive paralytic dilatation) by the increased tone of more proximal arteriolar segments can be achieved either by a nervous or by

a musculo-endocrine mechanism.[6] If the latter intervenes predominantly, as in the case of crush-wound syndrome, irreversible changes such as arteriolosclerosis are inevitable, and the chain of events described in the textbooks for this vascular lesion is bound to unfold if the patient lives long enough.

I believe that the notion of the existence of a musculo-endocrine regulation of the arteriolar tone will prove useful in the study of hypertensive diseases and arteriolosclerosis.

The experimental data available so far seem to confirm the early onset of renal vasoconstriction in crush wounds. Keele and Slome[16] observed a fall of the blood pressure and a marked reduction of renal blood flow immediately following the release of complete ischemia of a limb maintained for 4 to 5 hours in anesthetized cats. This reduction was more marked than in animals in which the blood pressure was lowered to the same extent by hemorrhage. In these early stages, however, vasoconstriction seems to involve only the post-glomerular arterioles, for Eggleton, Richardson, Schild, and Winton[17] observed glomerular congestion in the dog during the first 48 hours. The blood casts observed by Duncan and Blalock[18] in similar experiments also point to a condition of stasis in the glomerular tufts in the early stages. As generally accepted, the constriction of the post-glomerular arterioles is one of the normal mechanisms regulating glomerular circulation and filtration (Smith,[19] Richards[19a]), but obviously, when it is excessive, it impairs both glomerular and tubular functions. This seems to happen in the early stages of experimental crush wounds (Eggleton *et al.*[17]). My observations on post-mortem material suggest that in human patients surviving 4 to 9 days, vasoconstriction extends from the post-glomerular arterioles to the glomerular arterioles to the glomerular tufts. This would explain why pigment casts are eliminated with the urine in the early stages and retained in the kidneys towards the end: in the beginning, the flow of urinary filtrate from congested glomeruli is strong enough to wash away the casts; later on, when the glomeruli remain ischemic for long periods, the flow becomes too weak to do so.

I believe that renal deficiency observed in the crush syndrome is the result of vasoconstriction involving first the post-glomerular arterioles and later the glomerular tufts. Vasoconstriction can be followed by paralytic dilatation (as in cases A-1 and A-5). The gradual rise of the blood pressure seems to be caused by the increased vasopressive endocrine function of the pre-glomerular arteriolar wall following the deterioration of the circulation in the tufts, in the post-glomerular arterioles, and in the intertubular capillaries.

The leakages in the distal segments of the tubules (Dunn *et al.*,[1]

Bywaters and Dible [2]) probably lower the urinary output still more. In discussing the urinary function, glomeruli and tubules must not be considered independently. The existence of a connection at the level of the macula densa suggests a possible functional relationship between the two parts of the nephron.[7] For the time being I cannot say definitely whether the arteriolar constriction is the result of a direct and early impact of a substance of muscular origin on the arterioles or is an indirect one caused by the altered glomerular filtrate and by the damaged renal tubules so closely connected with the glomerular arterioles. In favor of the first of these two possibilities is the fact that, from the onset, peripheral vasoconstriction has usually been observed in crush-syndrome patients. Moreover, the casts and tubular lesions can be the effect of a primary severe ischemia of the kidney (Scarff and Keele [20]).

The patches of subendothelial transudate in the pre-glomerular and post-glomerular arterioles, present in all cases examined, may have existed prior to the muscular injury or may have been caused by it. If the former interpretation is correct, it would mean that incipient renal arteriosclerosis predisposes to the severe vasoconstrictive effect of ischemic muscular necrosis. But the second interpretation is more plausible because severe hypohemia, by increasing considerably the permeability of the endothelium, induces the formation of patches of transudate stainable with aniline dyes in the Goldblatt experiment.[7] Moreover, in crush-wound kidneys the presence of degenerated granular cells in the transudate indicates that the latter is of recent date. In my recent monograph[7] I have given the reasons in support of the opinion that the vasopressive substance is formed to a great extent within the wall of the renal arterioles as the result of the activation of transuded plasma globulin by the glandular product of the endocrine muscle cells. Braun-Menendez and Page (cited by Houssay and Dexter [21]) considered only the action of renin on plasma globulin in the blood stream. Incipient arteriosclerosis should be considered as a deviation of this intraparietal activation of plasma globulin.

Why are the intertubular capillaries filled with intact erythrocytes in spite of the deficient glomerular circulation? The blood is supplied to them by the subcapsular capillary network and by pre-glomerular Ludwig branches. In respect to the Ludwig branches it may be useful to give more information. In a child, 12 years of age, who died from subacute glomerulonephritis, the vasa afferentia and efferentia of nine deep-seated glomeruli were reconstituted; from the vas afferens of two of them a large Ludwig arteriole branched off and supplied capillaries in the cortex as well as in the medulla. Figure 1 of my paper on renal

ischemia [4] gives an appropriate idea of the frequency of these arterioles in the kidney of the dog.

Though fairly numerous in the deeper zone of the renal cortex, these branches do not seem to supply enough blood to restore the intertubular circulation to normal in cases of crush wound. There are indications of a sluggish circulation in some parts of the intertubular capillary network, especially around the glomeruli (accumulation of monocytes and polymorphonuclear leukocytes, stainable blood-plasma). The slowing down of the blood stream is probably one of the causes of the interstitial edema, the other being leakages in the distal part of the nephron.

The renal vascular changes under discussion are identical with those of cases labeled as "traumatic uremia" in which muscle laceration (*e.g.*, by shell splinters) is accompanied by severe wound infection (as in cases 76 and A-12-B), and also with those of eclamptic anuria [5] and acute glomerulonephritis. In the two latter conditions the existence of arteriolar spasms is generally agreed upon. In other words, the renal lesion of the crush syndrome resembles that in acute glomerulonephritis and the vascular reaction considered in this paper is perhaps allergic. It is interesting to note that in cases of eclampsia and fulminating acute glomerulonephritis in which the increase of the granular, glandular formation of the afibrillar cells is so striking, significant amounts of renin have been detected in the blood (Dexter and Haynes [15]).

The vasculo-nephrotoxic substance released by autolysis of the muscle tissue is probably some early intermediate breakdown product of a large protein molecule formed under strictly anaerobic conditions (Eggleton [22]), which is possibly cytotoxic. This may account for the frequent observation of centrolobular necrosis of the liver in fatal crush wounds (Bywaters and Dible [2]) and in cases where severe aerobic infection of lacerated muscle favors anaerobic autolysis after exhaustion of the oxygen (case 76). In mentioning these hepatic lesions it must be kept in mind that the liver is the probable source of hypertensinogen.

Although I cannot make any conclusive contribution to our knowledge regarding the nature of the substance which interferes with the tonus of the renal arterioles in cases of crush wound, it may be worth while to report the fortuitous observation which follows: In a rabbit undergoing a chronic "Goldblatt experiment," rupture of the psoas muscle accompanied by a large hematoma occurred accidentally; this lesion was followed 48 hours later by a very marked centrolobular hepatic necrosis and incipient circulatory disturbances in the glomeruli

of the kidney. This observation suggests that the nephrotoxic substance is not myohemoglobin (as tentatively proposed by Bywaters and Stead [23]) since this substance does not exist in the skeletal muscles of the rabbit.

A systematic investigation of the effect of anaerobic autolysates of muscle tissue and of the resorption of large hematomata on the endocrine cells of the renal arterioles may open an interesting field for further research.

Summary and Conclusions

1. In six cases, evidence was found to indicate that renal deficiency in the "crush syndrome" and "traumatic uremia" is to a great extent the result of vasoconstriction (followed by paralytic vasodilatation), involving first the *post-glomerular* arterioles and later the glomerular tufts.

2. As in the Goldblatt experiment, it is suggested that the gradual increase of the blood pressure observed in these cases may be accounted for by a striking increase of the afibrillar cells of the media of the pre-glomerular arterioles, which acquire cytologic features of glandular activity and are considered to participate in the formation of a vasopressive substance.

3. Subendothelial patches of freshly coagulated blood-plasma are constantly found in the pre-glomerular and post-glomerular arterioles. These patches are found in the vicinity of granular afibrillar cells which show signs of incipient regression. It is suggested that this may be evidence of an interaction of the blood-plasma and the hormone produced by the afibrillar cells, within the arteriolar wall.

4. The glandular transformation of the media of the pre-glomerular arterioles follows circulatory disturbances in the glomeruli and more peripheral vessels. It is suggested that this transformation is the morphologic expression of a vasopressive compensatory reaction which, in favorable conditions, can restore the deficiency of the more peripheral circulation.

5. The renal lesions observed in fatal crush wounds or traumatic uremia are identical with those of eclamptic anuria and fulminating glomerulonephritis.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

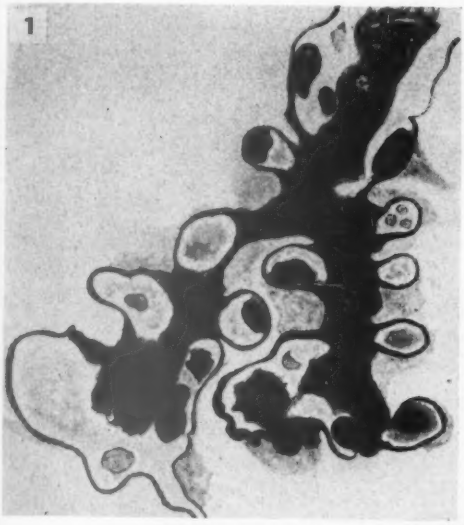
PLATE 87

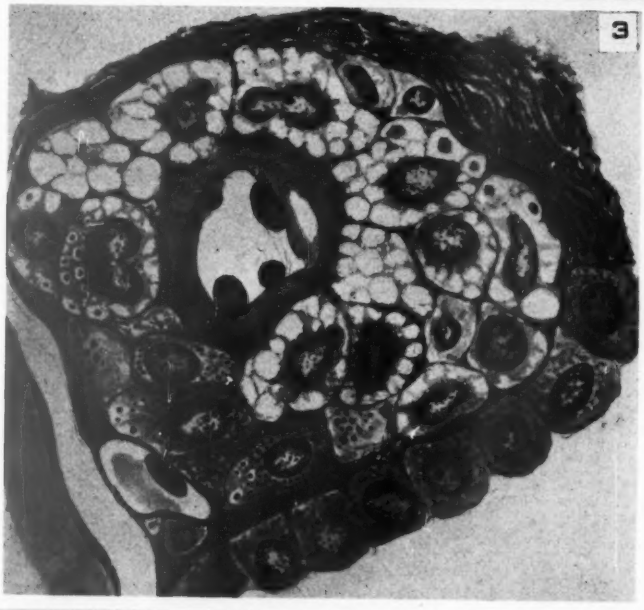
FIG. 1. Ischemic glomerular tuft (case A-1, crush wound). Longitudinal section of part of the mesangium supporting the loops, showing increase of the collagen. Cell expansions of the mesangium cells have disappeared. In the loops, some of which are retracted and others relaxed, may be noted coagulated plasma (after fixation), shrunken, hemolyzed red blood cells, one enlarged endothelial cell, and a cluster of thrombocytes. Fixative, 10 per cent formalin. Masson's trichrome stain.

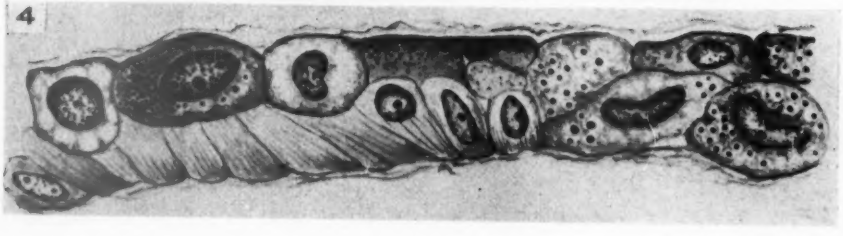
FIG. 2. Juxta-glomerular apparatus seen in longitudinal section and connected with the macula densa (case A-1, crush wound). The microcellular constituent is composed of spindle-shaped cells and is situated in the angle formed by the glomerular arterioles. The macrocellular constituent is composed of afibrillar cells and has a variable cytologic aspect (granular or vacuolar, or in regression). There is fresh subendothelial vacuolar transudate. Shrunken, hemolyzed red blood cells are seen in the infundibulum of the glomerulus. Fixation, 10 per cent formalin. Masson's trichrome stain.

FIG. 3. Juxta-glomerular apparatus seen in transverse section and connected with the macula densa (case A-5, crush wound), showing intense vacuolation of the granular afibrillar cells, vacuolar complexes (Dunihue and Candon [13]), and fresh subendothelial transudate. A capillary may be seen close to the group of granular cells. Fixative, 10 per cent formalin. Masson's trichrome stain.

FIG. 4. Tangential section of a pre-glomerular arteriole in the outer zone of the renal cortex (case 76, traumatic uremia observed in 1914–18). There is a granular transformation of the media. Afibrillar muscle cells show variable cytologic features and contrast with the ordinary smooth muscle cells. Bouin's fixative. Masson's trichrome stain.







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Renal Arteriolar Changes in Crush Syndrome

PLATE 88

FIG. 5. Juxta-glomerular apparatus seen in longitudinal section and connected with the macula densa (case 76). There is hypertrophy of the afibrillar cells, some of which contain coarse siderophilic granules. One afibrillar cell protrudes into the lumen of the arteriole. Glomerular ischemia and interstitial edema are present. Flemming's fixative. Heidenhain's iron hematoxylin stain.

FIG. 6. Juxta-glomerular apparatus in tangential section (case A-4, crush wound), showing considerable hypertrophy and granularity of the afibrillar cells, cellular increase in the wall of the infundibulum, and vacuolation of the cells of the macula densa. The contrast may be noted between the latter cells and the epithelium of the proximal convoluted tubule. Fixation, 10 per cent formalin. Masson's trichrome stain.

FIG. 7. Case A-1. Intertubular capillaries, some showing stainable plasma. Interstitial connective tissue is edematous. Fixation, 10 per cent formalin, Masson's trichrome stain.

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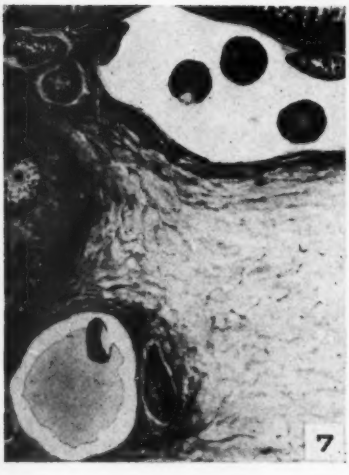
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STUDIES OF THERMAL INJURY

Parts I, II, and III of Studies of Thermal Injury by A. R. Moritz, F. C. Henriques, Jr., and associates will appear in *The American Journal of Pathology* as follows:

I. Henriques, F. C., Jr., and Moritz, A. R. Studies of Thermal Injury. I. The Conduction of Heat to and through Skin and the Temperatures Attained Therein. A Theoretical and an Experimental Investigation. This issue, pages 531–549.

II. Moritz, A. R., and Henriques, F. C., Jr. Studies of Thermal Injury. II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns. September, 1947.

III. Moritz, A. R. Studies of Thermal Injury. III. The Pathology and Pathogenesis of Cutaneous Burns. An Experimental Study. November, 1947.

—*Editor*

STUDIES OF THERMAL INJURY

I. The Conduction of Heat to and through Skin and the Temperatures Attained Therein. A Theoretical and an Experimental Investigation *

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I. Introduction

Never before in the history of man has heat energy been so important a cause of disability and death as during World War II. The need for precise knowledge regarding the thermal tolerances of living tissue and the nature of the cellular and somatic changes induced by hyperthermia was felt by those responsible not only for the protection and medical care of our own military personnel but also for the development and use of such thermally effective weapons as the flame thrower, the atomic bomb, and various other incendiary or explosive missiles.

Although thermal injury has always constituted a problem of medical importance, the requests of various branches of the Armed Forces for precise and quantitative information disclosed a dearth of basic facts relating to the casualty-producing effectiveness of heat energy. Remarkably little information was available concerning the mechanism by which hyperthermia leads to irreversible cellular injury, the reciprocal relationships of time and temperature in the production of either cutaneous or systemic injury, the relationship between environmental heat, surface temperature and the slope of the transcutaneous thermal gradient, the pathogenesis of cutaneous burns, or the physiological mechanisms by which external heat may be responsible for acute disability or death.

In an attempt to elucidate some of these problems and to satisfy some of the more pressing needs of the Armed Services for quantitative data, a series of studies was undertaken. It became apparent that the information thereby acquired was of such fundamental importance to an understanding of the problem of thermal injury from a civil as well as from a military standpoint that its publication in the open literature was authorized.

* This work has been done in part under contract NDCrc-169 between the President and Fellows of Harvard College and the Office of Scientific Research and Development, and in part under subsidy from the Medical Division, Chemical Warfare Service, through a contract with New York University, New York City. Neither the Office of Scientific Research and Development nor the Medical Division, Chemical Warfare Service, assumes responsibility for the accuracy of the statements contained herein.

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This report, which is the first of a series, is concerned in part with a general consideration of the physical laws that relate to heat transfer and temperature change and in part with a quantitative experimental study of the rate of heat transfer through skin at different surface temperatures and the effects of different rates of energy transfer on the subsurface temperature gradient.

II. Theoretical Considerations

THE NATURE OF HEAT

The concept of temperature arises from the sensations of hotness and coldness. Experience has shown that when a group of substances of different temperature are kept free of outside disturbances, the hotter bodies will get colder and the colder bodies hotter, and, ultimately, these substances will reach a state of complete thermal equilibrium (identical temperature). The hotter bodies are said to have lost heat and the colder bodies are said to have gained heat. This concept of heat becomes quantitative by defining a unit of heat, the calorie, as the amount of heat gained by 1 gm. of liquid water under atmospheric pressure when the temperature increases from 14.5° to 15.5°C.

A gain in heat, which is discernible through a rise in temperature, is associated with an increase in the intra-molecular and inter-molecular motion. Thus, heat can be considered as the energy stored in a substance by virtue of the state of its molecular motion. Certain manifestations of this increase in energy are readily observable; for example, melting, vaporization, decomposition, and alteration in rate of diffusion and chemical reaction.

Beside the definition of a calorie, there are other physical concepts pertaining to heat which are requisite to an understanding of the general problem of thermal injury, namely, heat capacity and heat transfer.

Heat Capacity, C_p

Heat capacity, or specific heat, of a substance is the amount of heat which is required to raise its temperature by 1°C.

The importance of heat capacity in relation to thermal injury is readily seen by considering the respective propensities for production of injury of 1 gm. of water ($C_p = 1.00$) and 1 gm. of silver ($C_p = 0.06$) both at 100°C. and in contact with 1 gm. of thermally insulated skin ($C_p \approx 0.7$) at 35°C. After equilibrium is reached in the former case, the temperature of the skin is increased to 73°C., whereas in the latter case it is increased only to 42°C.

It is apparent that if the skin were to equilibrate rapidly enough

when placed in contact with a hot body, there is insufficient heat in 1 gm. of silver at 100°C. to produce injury to 1 gm. of skin. Actually, of course, the skin, due to its thermal insulating properties, does not equilibrate rapidly enough and the portion of skin nearest the silver does reach a sufficiently high temperature to produce injury before thermal equilibrium is reached. Hence, another physical property of importance in the problem of thermal injury is heat transfer.

Heat Transfer

In certain experiments to be described in ensuing articles,[1,2] heat was transported to the skin by three methods: convection, radiation, and conduction. With convection and radiation,[1] heat reached the skin under such circumstances that the uptake of heat was primarily determined by the heat source. With conduction,[2] the amount of heat absorbed by the skin was primarily determined by the properties of the heat absorber, the skin itself.

Convection. Convection is the mechanism by which hot air transports heat to a cooler surface due to the eddying currents that arise. The air velocities of the eddy currents are about 1.6 km. per hour. An equation has been developed for the transfer of ambient heat by natural convection from a large envelope of hot air surrounding cylindrical objects about 30 cm. in diameter.[3,4] This equation shows that q, the caloric uptake per minute per sq. cm. of surface, can be expressed as follows:

$$q = 0.0026\,(T_a - T)^{\frac{5}{4}} \qquad (1)$$

where T_a is the air temperature in °C. and T is the surface temperature in °C. Thus, using a skin temperature of 40°C., air at 100°C. and at 400°C. will transport to the skin about 0.4 and 4 calories per sq. cm. per minute, respectively. It is also apparent that as this heat is absorbed by the skin, the surface temperature of the skin will rise and the caloric uptake of the animal will decrease with time.

It is of interest to compare the caloric uptake rate of skin at 40°C. when an atmosphere of steam maintained at 100°C. is substituted for the air. Under these conditions, about 300 calories per sq. cm. per minute would be absorbed by the skin,[3] if the surface temperature could be maintained at 40°C. This 800-fold increase in caloric bombardment as compared to air is due to the latent heat of condensation of steam. This, of course, is why steam is an enormously greater hazard than hot air in the production of heat injury.[5]

Radiation. All substances give off heat in the form of radiant energy

in amounts that are predetermined by their surface temperatures. When this radiation impinges upon another body a certain fraction is absorbed and is changed into heat. Thus, if two substances at different temperatures are placed in an enclosure, there is a continual exchange of energy; the hotter body radiating more energy than it absorbs and the colder body absorbing more heat than it radiates. In the special case of an animal completely enclosed in a large box of source temperature, T_r, in °C., the caloric uptake rate, q, of the animal, due to this interchange of radiant energy between the skin and the wall of the box, is expressed by the following equation: [3,4]

$$q = sef\,[(T_r + 273)^4 - (T + 273)^4] \qquad (2)$$

where s is the radiation constant and is equal to 8.2×10^{-11} calories per sq. cm. per T^4 per minute, e is the effective emissivity of the hot walls of the box, and f is the absorptivity of the skin to radiation emitted at T_r. Under experimental conditions to be described, the product, ef, can be taken as about 0.8. Thus, when the skin temperature is 40°C., the hot walls at 100° or 400°C. will radiate to the skin about 0.7 or 13 calories per sq. cm. per minute, respectively.

Conduction. Conduction is defined as the transfer of heat from the hotter portion of a substance to a colder portion of the same substance, or from a hot body in physical contact with a cold body, when in each case there is no appreciable displacement of any of the molecules comprising these substances. It is this restriction that differentiates conduction from convection.

In certain experiments to be described, heat was conducted from either a hot solid or a hot liquid [2] to the skin. In these experiments, the purpose of both the solid and liquid heat source was to maintain the temperature of the skin surface at a predetermined constant value and hence the conduction of heat through the heat source need not be considered. In hot air experiments,[1] thermal conduction through air is small as compared to convection, and this small contribution is included in equation 1. Thus, conduction of heat through the skin only need be considered.

In all cases of heat flow by conduction, a temperature gradient must exist within the substance. If this temperature gradient varies with time, the rate of heat flow will also vary with time. This type of heat flow in which temperature is a function of both position within the body and time is called heat conduction in the unsteady state. Heat conduction in the steady state refers to all cases in which the temperature at any point within a substance does not depend upon time. Under these conditions the amount of heat flow through the medium is

determined by this temperature gradient and by the ability of the body to conduct heat (thermal conductivity). It is the latter case which will be considered first. The equation for steady state heat conduction inside a rectangular homogeneous body is based upon Fourier's law [3,4,6] and is given by:

$$q = \frac{K}{L}(T_1 - T_2) \tag{3}$$

where K, the thermal conductivity, is expressed in calories per minute per sq. cm. perpendicular to the direction of heat flow per unit temperature gradient, °C. per cm. length of path. L is the path length through which the heat flows and T_1 and T_2 are the temperatures in °C. at the beginning and end of the path respectively; q has been previously described.

This equation makes possible the experimental determination of the *in vitro* thermal conductivity of the four respective sections of cutaneous and subcutaneous tissues: epidermis, dermis, fat, and muscle, and also of any combination thereof.

GENERAL THEORY OF HEAT FLOW THROUGH SKIN

By making use of the above brief definitions of the various physical factors involved in the transport of heat to and through the skin, it is possible to consider how the application of heat affects the time-temperature relationship within a given cutaneous site. It is apparent that in order to make heat flow inward from the skin surface it is necessary to raise the temperature of the skin surface to an extent that overcomes the normal existing gradients. This can be accomplished by means of an external source of heat through conduction, convection, or radiation. Once the temperature of the skin surface is sufficiently high, the heat will start to flow inward, resulting in a general rise in temperature within the skin site.

This initial heat flow inward, *and thus the rate of temperature rise within,* will depend primarily upon two physical factors: (a) the heat capacity of the skin or the ability of the skin to absorb the heat, and (b) the thermal conductivity of the skin or the ability of the skin to transport the heat. After a certain interval of time the amount of heat entering the skin site will be balanced by the amount of heat leaving the skin site, and the skin will be "heat saturated." In this state, the new temperature distribution within the skin site will become nearly invariant with time, and the amount of heat flowing through the skin will depend only upon (b) and the skin surface temperature.

It is to be recognized that the above picture involves not only the so-

lution of the steady state of heat conduction, but also the solution of the initial unsteady state of heat flow. In order to solve even the above "idealized" picture, it would be necessary to know the initial temperature gradients within the tissues, the thicknesses, densities, thermal conductivities, and heat capacities of the various layers, and the skin surface temperature as a function of time.

The solution of such a problem involves the following Fourier heat equation:[6]

$$(4) \qquad \frac{K}{PC_pL^2}\left(\frac{d^2T_{xt}}{dx^2}\right) = \frac{dT_{xt}}{dt}$$

where T_{xt} is the temperature at the time, t, at a distance, x, within the skin measured from the skin surface. P is density, and the remaining symbols have been previously defined.

The solution of equation 4, subject to the above-mentioned conditions, is exceedingly complicated. Yet, superimposed upon this are the numerous indeterminate *in vivo* factors which arise when we go from the "idealized picture" to the living animal. It is useful to enumerate the most important of these various indeterminate factors:

(a) Site variations in the respective thickness of epidermis, dermis, fat, and muscle.

(b) Variation of existing temperature gradients within the skin with respect to time and/or position of site.

(c) Unknown average rate of blood flow through the various skin layers, and the unknown variations of the unknown rate of flow with respect to position of site and temperatures within the site.

(d) The appearance of edema fluid in variable quantities which brings forth indeterminate alteration in the density, heat capacity, thickness, and thermal conductivity of the various layers of skin so affected.

It is obvious from the above discussion that any general solution of the time-temperature relationship within a skin site, when heat is applied, is not possible. However, with certain of the experiments to be described in detail,[1,2] it is possible *to derive to a first approximation the time-temperature relationship in the layer of basal epidermal cells.** These experiments were either (i)[2] conducted so as to bring the skin surface immediately to, and maintain it at, a predetermined temperature level until the threshold of irreversible epidermal injury was reached; or (ii)[1] the entire animal was surrounded com-

* Actually, these time-temperature relationships can also be estimated at either the skin surface or at any distance within the epidermis. The basal epidermal layer has been specifically chosen since increases in temperature of these cells are of the most import in the production of epidermal injury by heat.

pletely by an envelope of ambient (air) and radiant heat. These experimental conditions at the boundary of the skin surface and source of heat are expressed by the following equation:

$$q = H(T_s - T) \tag{5}$$

where q and T have been previously defined (equations 1 and 2). T_s is the temperature of the heat source in °C. and H, in calories per sq. cm. per minute, is known as the heat transfer coefficient. Conditions under experiments i were tantamount to an infinite heat transfer coefficient ($H = \infty$); and with experiments ii the heat transfer coefficient is finite and the numerical value is readily obtained by combining the radiant and ambient contributions to heat transfer coefficient as computed by equations 1 and 2, respectively. In order to solve equation 4 under the boundary condition expressed by equation 5, it is necessary to assume that the ratio of the total tissue thickness to the epidermal thickness (about 80 μ) is infinite rather than finite. This assumption will lead to slightly longer time intervals for "heat saturation" of the epidermis than are to be experimentally expected. The integration [6] of equation 4 under the above conditions results in equation 6:

$$\frac{T_s - T_t}{T_s - T_o} = \theta\left[\frac{\gamma}{\sqrt{t}}\right]\left\{ e^{\frac{HL}{K}\left(1 + \frac{HLt}{4\gamma^2 K}\right)} \right\}\left\{ 1 - \theta\left[\frac{\gamma}{\sqrt{t}}\left(1 + \frac{HL}{2\gamma^2 K}\right)\right]\right\}$$

where

$$\theta[Y] = \frac{2}{\sqrt{\pi}}\int_0^Y e^{-x^2}dx \tag{6a}$$

and γ is computed by means of equation 6b.

$$\gamma = \frac{L}{2\sqrt{\frac{K}{PC_p}}} \tag{6b}$$

T_t is the temperature of the basal epidermal cells at the time, t, in seconds. T_s is the temperature of the heat source. T_o is the temperature of the skin surface previous to the exposure to heat. L is the distance of the basal cells from the skin surface. P is the density of the basal epidermal layer. The other symbols have been previously defined and are experimentally determinable. The integral that defines $\theta[Y]$ (equation 6a) is respectively equal to $\sqrt{\pi}/2$ and zero when Y is infinite ($t = 0$) and Y is zero ($t = \infty$). For other values of Y, the numerical value of the integral is tabulated.[7]

The time-temperature relationships at the basal-epidermal layer

during an exposure of the animal to a source of constant ambient and radiant heat will be evaluated by means of these equations in section IV.

In experiments in which the skin surface was brought immediately to, and maintained at, a predetermined constant temperature, H, the heat transfer coefficient, is nearly infinite, and equation 6 reduces to

$$\frac{T_s - T_t}{T_s - T_o} = \theta\left[\frac{\gamma}{\sqrt{t}}\right] \tag{6c}$$

where, as before, γ is given by equation 6a. It is to be noted that in this case T_s can be taken as the skin surface temperature during the entire heat exposure, since the temperature of the heat source is identical with the surface temperature once heat exposure begins.

Equation 6c results in a basal layer temperature which becomes, after a certain time interval, essentially identical with the skin surface temperature. Actually, there will always exist a small but finite temperature gradient between the surface and the basal cell layer. This steady state gradient can be experimentally determined by means of equation 3, and the true temperature of the basal layer can be quite accurately computed for any time, *t*, by using equation 6c until the steady state temperature obtained through equation 3 is reached. Computations using equation 6 to ascertain basal epidermal temperatures will be given in section IV.

III. An Experimental Investigation of the Quantities Involved in Both the Steady and Unsteady State of Heat Conduction Through the Skin

Determination of the Heat Capacity of the Four Pertinent Tissues

The apparatus used for the determination of the heat capacity of these cutaneous and subcutaneous tissues need not be described in detail, since the well known method of mixtures was used.[8] Briefly, this procedure consisted of heating a known weight (about 10 gm.) of each tissue in a thin brass container and rapidly dropping it into a water calorimeter. The heat capacity of the tissue was readily computed from temperature rise of the water as measured by a Beckmann thermometer.

In order to obtain pure epidermis for these determinations, the following method was used. After the hair was shaved as closely as possible, the pig was immersed in the water at 55°C. for about 1

minute, then withdrawn and the skin carefully dried. It was then possible to remove strips of pure epidermis by scraping with a knife. The remaining tissues were readily obtained in a relatively pure state by dissection.

The values of the heat capacities of the respective tissues of two pigs are given in Table 1.

TABLE I

Heat Capacity of Cutaneous and Subcutaneous Tissues of the Pig in Calories per Gram per °C.

	Epidermis	Dermis	Fat	Muscle
Heat capacity, C_p	0.887 0.845	0.785 0.753	0.538 0.573	0.890 0.926
Average value, $\overline{C_p}$	0.86	0.77	0.55	0.91

In view of the known similar heat capacities of dry tissue, the variations of the different tissues as shown in Table 1 are probably due to the water content. In this respect, the high value for epidermis (0.86) is understandable since it was found experimentally that the water content of epidermis, in spite of the presence of the cornified layer, averaged about 76 per cent.

Determination of the Thermal Conductivities of Cutaneous and Subcutaneous Tissues of the Pig

The experimental determinations of the thermal conductivities of cutaneous and subcutaneous porcine tissue were based on equation 3. The respective tissues were placed on a copper cylinder, 5 cm. in diameter and 10 cm. high. The temperature of this tissue-cylinder interface was measured by means of an iron-constantan thermocouple soldered into the face of the copper cylinder and a type K2 potentiometer.* The automatic recording caloric applicator[9] was then placed over, and in contact with, the exposed face of the tissue. This apparatus provided a means of automatically recording the vertical rate of caloric flow from the applicator through the tissue to the copper cylinder as functions of the temperatures of both tissue surfaces. Thus, when the tissue became "heat saturated," knowledge of the caloric input into the tissue, of the temperatures of the tissue-applicator (about 48°C.) and of tissue-cylinder (about 30°C.) interfaces, and of the thickness of the tissue made possible computation of thermal conductivity. The average tissue thickness was determined

* Leeds & Northrup, Philadelphia, Pa.

by measuring the distance between the face of the applicator and the face of the cylinder. The thermal conductivities of all tissues except epidermis were obtained by this procedure, since in view of the thinness of epidermis the above method was not feasible.

The method of difference was used with epidermis. A section of well shaved skin tissue consisting of dermis and epidermis was rigidly clamped to the copper cylinder, water at 55°C. was poured over the skin, and the excess water was removed by blotting. The clamps prevented lateral contraction of the heated tissue and the hot water facilitated subsequent removal of the epidermis. The conductivity determination was then made, the epidermis scraped off, and the determination repeated. As a further check, in certain experiments a

TABLE II

In Vitro Thermal Conductivities, K, of Pig Tissues

$$K = \frac{\text{calories} \times \text{cm.}}{\text{cm.}^2 \times \text{minutes} \times \text{°C.}}$$

	Epidermis	Dermis	Subcutaneous fat	Subcutaneous muscle
K	0.036	0.054	0.021	0.064
K	0.023	0.053	0.024	0.062
K	0.032	0.051	0.023	0.073
K	0.03	0.053	0.023	0.066

strip of intact epidermis was placed over the denuded dermis and the measurement repeated. The thickness of numerous pig epidermal strips was determined with a micrometer. The thickness was about $80 \pm 10\ \mu$.

At least triplicate determinations were made on each of the four tissues of three different pigs (about 10 kg.). The average values of the thermal conductivities obtained are given in Table II.

In view of the thinness and uncertainty as to thickness of the epidermis, the wide variation in the epidermal thermal conductivity was to be expected. The data pertaining to the other tissues were considerably more reproducible.

It is of interest to compare some of these data with those of Breuer,[10] who determined the respective thermal conductivities of both muscle and fat of cow, horse, pig, and dog. This investigator found that the conductivities of pig muscle and fat, expressed in the above units, were 0.060 and 0.021, respectively; furthermore, essentially the same values were found for the muscle and fat of the other three animals. In view of the excellent agreement between Breuer's value and ours for pig muscle and fat, it is difficult to understand the value, 0.03, that Hardy and Soderstrom [11] reported for both cow muscle and fat. Un-

fortunately, no description of their experimental method was given. In order to investigate this discrepancy, the thermal conductivity of beef muscle was redetermined and an average value of 0.057, which agrees with the results of Breuer, was obtained.

In view of the numerous indeterminate factors (section II) which enter into the *in vivo* conduction of heat through pig skin, the *in vitro* thermal conductivities of these four tissues are not of themselves very useful. However, they do serve as a baseline in the interpretation of certain experiments to be described.

In Vivo Observations of Caloric Uptake of Pig Skin and the Rise in Temperature at the Dermis-Fat Interface as a Function of Both Time and Skin Surface Temperature

It was of interest to ascertain the caloric uptake of the skin when the epidermal surface was maintained at various temperature levels between 45° and 100°C. Numerous such experiments have been performed and, as was to be expected (see section II), the data varied widely and were extremely difficult to interpret in detail. Therefore, only a small fraction of these data will be reported and the variations to be expected will be indicated.

During these experiments the temperature at the dermis-fat interface also was ascertained. A pig under nembutal anesthesia was clipped and shaved. The no. 27 gauge needle thermocouple [9] was introduced laterally into the dermis-fat interface in the following manner: Through experimentation it was found possible to insert laterally a no. 22 gauge trocar along the natural cleavage plane of the dermis-fat interface, until a point directly underneath the surface area to be exposed to heat was reached; then the no. 27 gauge thermocouple needle was inserted into the no. 22 gauge trocar until skin resistance could be detected. The no. 27 gauge needle was then withdrawn about 1 cm. The temperature (millivolts) of this needle couple was measured either intermittently with a type K2 potentiometer * or continually with a photo-electric recording potentiometer.†

After the determination of the skin temperature at a chosen site by means of the fine wire thermocouple,[9] the automatic recording caloric applicator [9] was applied, and a continuous record of the caloric uptake rate of the skin at a predetermined epidermal surface temperature was obtained.

After the heat exposure was terminated, the skin was cut to expose the hypodermic needle thermocouple and the distance from the dermis-

* Leeds & Northrup, Philadelphia, Pa.
† General Electric Co., Schenectady, N. Y.

fat interface to the skin surface was ascertained with a depth gauge. This depth before the application of heat was ascertained by control experiments on neighboring sites.

Caloric Uptake Rate of Pig Skin

Typical caloric uptake data as a function of time and epidermal surface temperature are presented in Table III. These data were a

TABLE III

A Guide (± 30 Per Cent) to the Caloric Uptake of Pig Skin as a Function of Time and Surface Temperature as Determined by the Automatic Recording Caloric Applicator[9]

Time interval	Skin surface temperature during heat exposure 45° C.	50° C.	55° C.	60° C.	65° C.
(*minutes*)	Caloric uptake rate of lateral thoracic skin in calories per minute per sq. cm.				
0 –0.2	6.0	9.5	12.0	15.0	17.0
0.2–0.4	2.2	3.7	4.9	6.9	8.4
0.4–0.6	1.8	2.8	3.8	5.6	6.7
0.6–0.8	1.7	2.5	3.1	5.1	5.9
0.8–1.0	1.6	2.3	2.7	4.7	5.4
1 –1.5	1.5	2.0	2.6	4.3	4.8
1.5–2	1.4	1.8	2.4	4.1	4.5
2 –3	1.2	1.6	2.3	3.7	4.2
3 –5	1.1	1.5	2.1	3.2	3.8
5 –7	1.0	1.4	2.0	2.8	3.5
7 –10	0.9	1.3	1.9	2.5	3.2
	Total caloric uptake in calories per sq. cm.				
0 –1	2.7	4.2	5.3	7.5	8.7
0 –5	7.5	10.7	14.3	21.8	25.2
0 –10	12.2	17.4	24.0	34.9	42.7

composite of at least three determinations on the lateral thoracic area of different pigs; five pigs in all were used. As was expected, in view of the numerous factors that determine the caloric uptake in a living animal (section II), the experimental variations inherent in duplicating exposures were considerable. Thus, these data served only as a rough guide (± 30 per cent) to the caloric uptake rate of pig skin.

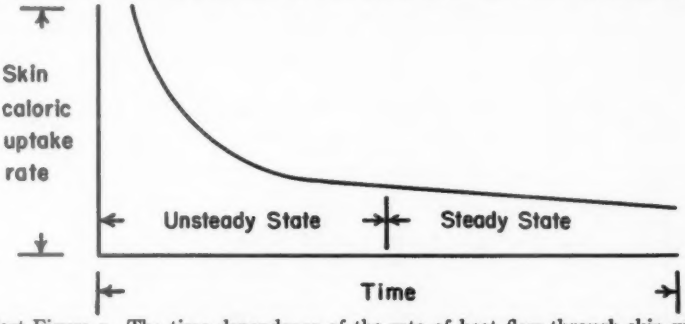
It was noted that the average caloric uptake rate of pig skin during the first 0.2 minute was about six-fold greater than the average caloric uptake rate during the steady state period (7 to 10 minutes). This six-fold difference was due to the initial necessity of heat saturating the tissue and was primarily a heat capacity effect. After the first few minutes the skin tissue was essentially heat saturated and the *in vivo* thermal conductivities of the various layers of pig skin primarily determined the caloric uptake rate.

When the data given in Table III are plotted against time, the curves obtained will conform in type to that shown schematically in Text-Figure 1. A mathematical analysis of the general form of these curves based on equation 5 showed that during the first 2 to 3 minutes of the heat application, the skin can be considered as an infinite body with a ratio of thermal conductivity to heat capacity that was approximately

the same as that computed from the *in vitro* determinations tabulated in Tables I and II. This agreement was probably due to the fact that the ratio of the thermal conductivity to heat capacity (equation 6b) was not nearly as sensitive to these indeterminate *in vivo* factors as the individual quantities themselves.

The Interface Temperature at the Junction of Dermis and Fat

The dependence upon time of the dermis-fat interface temperatures during exposure of the skin surface to a predetermined temperature between 45° and 90°C. is given in Table IV. These values were a composite of at least two experimental determinations on two different pigs (four determinations in all). As with measurements of the caloric



Text-Figure 1. The time dependence of the rate of heat flow through skin when the surface is immediately brought to, and maintained at, a constant temperature.

uptake, the variations in duplicating experiments were considerable, and these data serve only as a rough (± 20 per cent) guide to the time-temperature relationship at the dermis-fat interface. These data together with other experimental observations indicate the following:

(a) The junction of the fibrous dermis and the subdermal fat in the lateral thoracic area of a 10 kg. pig lay about 2 mm. below the skin surface. Ten minute exposures to surface temperatures of 50° to 70°C. increased, significantly, the thickness of the dermis. This increase in thickness was due to the accumulation of edema fluid in the dermis and the effect was maximal when the skin surface was maintained at about 60°C. Skin surface temperatures equal to, or greater than, 80°C. denatured the corium so rapidly that these mechanisms were destroyed.

(b) Although the continual caloric uptake by the skin tended to increase the dermal temperature, the appearance of relatively cool edema fluid tended to decrease it. At skin surface temperatures of 50° and 70°C. these two effects nearly counterbalanced, and after the first minute of heat exposure, the dermis-fat interface temperature remained essentially constant. With skin surface temperatures between 55° and 65°C. the rapid appearance of a large amount of edema

fluid more than compensated for caloric uptake, and the temperature at the interface between dermis and fat was temporarily lowered. This effect was maximal when the skin surface was maintained at about 60°C.

(c) When the skin surface temperature was maintained at 45°C., and probably at all other temperatures that fail to cause edema, the dermis became "heat saturated" after about 5 minutes of exposure.

TABLE IV

The Dependence on Time of the Dermis-Fat Interface Temperature (± 20 Per Cent) During Exposure of the Surface of Pig Skin to Predetermined Temperatures

Initial	Skin surface temperature in °C. 35.0	34.8	34.8	35.2	34.9	34.3	34.2	34.5
During exposure	45	50	55	60	65	70	80	90
Time	Dermis-fat interface temperature in °C.							
(*minutes*)								
	34.7	34.5	34.6	35.0	34.7	34.2	34.4	34.8
0.2	36	38	39	43	46	52	53	56
0.5	38	43	45	46	52	62	65	66
1.0	39.5	45	47	48	53	65.6	71	74
1.5	40	47	48	47	53	66.5	72	77
2.0	40.5	47	49	46	54	67	74	79
3.0	41	47	48.5	45	56	67.5	75	79
5.0	42	47	47.5	44.5	58	67.5	77	
7.0	42	46.5	47.5	47	58			
10.0	42	47	48	49	59			
Initial	Average thickness of corium in mm. 2	2	2	2	2	2	2	2
At termination of exposure to heat	2	2.5	3.2	4.2	3	2.5	2	2
	In vitro thermal conductivity of dermis at termination of exposure 0.06	0.1	0.09	0.10	0.16			

When edema fluid was produced, the time for dermal heat saturation was essentially indeterminate, but it apparently was not much greater than 10 minutes.

(d) Histological examinations showed that complete primary injury to the dermis immediately following heat exposure was obtained in all of these experiments when the skin surface temperature was maintained at 65°C. or above. The limited (five) time-temperature-injury data at the dermis-fat interface tended to indicate a quantitative relationship not at variance with that found for epidermal injury.[2,12]

(e) By making the reasonable assumption that the dermis was essentially "heat saturated" at the end of a 10 minute exposure, the *in vitro* thermal conductivities of dermis can be computed by substituting the

approximate caloric uptake (Table III), dermis-fat interface and skin surface temperatures, and the final dermal thickness in equation 3; neglect of the epidermal temperature drop introduced no appreciable error. Table IV also shows the results of these calculations. A comparison of these values with the experimentally determined *in vitro* value of 0.053 (Table II) for pig dermis indicates that the presence of edema fluid increased the thermal conductivity of dermis two- to three-fold. This increase in conductivity, however, was slightly more than compensated by the swelling of the dermis, and thus an edematous dermis was a somewhat better heat barrier to the underlying tissues than normal dermis. A comparison of the *in vivo* thermal conductivity of dermis obtained at 45°C. with the *in vitro* value tended to indicate that intact circulation probably increased the effective thermal conductivity of dermis by about 15 per cent.

IV. Estimation of the Temperature Changes at the Epidermal-Dermal Interface During the Exposure of the Skin Surface to Heat

In view of the thinness ($\sim$80 μ) of the pig's epidermis, experimental measurement of the time-temperature relationships at the epidermal-corium junction was not feasible. There are certain facts, however, that allowed the estimation of this time-temperature relationship with a considerable degree of certainty. In view of the extreme thinness of epidermis, the temperature of the basal layer was largely determined by skin surface temperature. This is most readily seen by solving heat conduction equation 3 for the steady state temperature of the basal epidermal layer. Of the four necessary experimental quantities; namely, skin surface temperature, epidermal thickness (about 80 μ), epidermal thermal conductivity (Table II), and caloric uptake of the skin at the requisite skin surface temperature (Table III), only the last two were subject to considerable variation ($\pm$ 30 per cent). Fortunately, even variations of this magnitude result in uncertainties of less than 0.2°C. in the steady state temperature of the basal epidermal layer.

Basal Epidermal Temperatures When the Skin Surface Is Immediately Brought to, and Maintained at, a Temperature between 45° and 100°C.

Before the steady state temperature is attained, the time-temperature relationship at the epidermal-dermal junction is given under these conditions to a good approximation by equation 6c, where γ has the numerical value

$$\gamma = 0.15 \tag{7}$$

if the time, t, is expressed in *seconds*.

The numerical constant, 0.15, is not subject to the experimental uncertainties of the quantities requisite to computation by equation 6b, since this value was empirically determined [12] with considerable accuracy from data on experimental temperature-time-epidermal injury.[2] An identical value for γ can be directly computed also by substituting into equation 6b the heat capacity, thermal conductivity, and thickness

TABLE V

The Computed Time-Temperature Relationships for the Epidermal-Dermal Interface When the Skin Surface Is Immediately Brought to, and Maintained at, a Specific Temperature

	Surface temperature during heat exposure				
	45° C.	55° C.	65° C.	80° C.	100° C.
Time	Temperature at basal epidermal layer*				
(*seconds*)					
0	35.0	35.0	35.0	35.0	35.0
0.01				36.3	37.0
0.02			38.9	40.9	43.4
0.05		41.8	45.2	50.3	57.1
0.1	40.1	45.2	50.3	57.9	68.2
0.2	41.3	47.6	53.9	63.3	75.9
0.5	42.7	50.4	58.1	69.6	85.1
1	43.3	51.6	60.0	72.4	89.1
2	43.8	52.6	61.4	74.6	92.3
5	44.2	53.5	62.7	76.6	95.1
10	44.5	53.9	63.4	77.6	96.6
30	44.7	54.4	64.1	78.6	98.0
60	44.8	54.6	64.4	79.0	98.6
120	44.9	54.9	64.5	79.4	99.2
300	44.9	54.9	64.7	79.5	99.3
600	44.9	54.9	64.8	79.7	99.6
Steady state†	44.8	54.5	64.2		

* Computed by means of equations 6b, 6c, and 7.
† Computed by means of equation 3 and experimental data of Table II.

of epidermis, and assuming an epidermal density of 0.8 gm. per cc., a most reasonable value. In view of the two completely independent methods, one of which was *in vivo* and the other *in vitro*, considerable confidence can be placed in the adaptation of the "infinite body picture" (section II) to the solution of the time-temperature relationship at the epidermal-dermal junction during the unsteady state period of heat flow.

The computation of the temperature of the basal cell layer of the epidermis as a function of both time and skin surface temperature is given in Table V. These data show that there was a rapid rise in the temperature of the basal epidermal layer when the skin surface was immediately brought to, and maintained at, a specified constant temperature. A comparison of the data of the unsteady state computed from equation 6c with those of the steady state obtained by means of

equation 3 shows that the epidermis under the above conditions became essentially "heat saturated" after a heat exposure of 0.5 to 1.0 minute's duration.

It must be re-emphasized that these data apply only to situations in which the heat transfer coefficient, H, from the temperature source to the skin surface can be considered as infinite. In all cases where H is finite, an analysis similar to that given below is required.

Basal Epidermal Temperatures When the Entire Animal Is Surrounded by an Envelope of Ambient (Air) and Radiant Heat between 80° and 175°C.

In the previous discussion, the time-temperature relationships at the epidermal-dermal junction depended only upon the rate of heat transfer through the skin and the constant temperature of the heat source. To this must now be added the slow rate at which heat is transported from its source to the skin surface via air conduction, air convection, and infra-red radiation. The mathematical solution of this problem is given by equations 6 and 7 where the only quantity that requires further consideration is H, the heat transfer coefficient from the heat source to the skin surface. This quantity is readily computed through the substitution of equation 1, heat transfer by convection, and equation 2, heat transfer by radiation, into equation 5. The numerical values of the heat transfer coefficient which were obtained at particular source or air temperatures are shown in Table VI. A comparison of these values of H, 0.015 to 0.026 $\frac{\text{calories}}{\text{sq. cm. per minute per °C.}}$, with epidermal thermal conductance, K/L (Table II), numerically equal to 4 in these units, indicates the slow rate at which ambient and radiant heat was transferred to the skin surface as compared to the rate at which this heat flowed through the epidermis.

Table VI gives also the estimated temperature of the basal epidermal layer as function of source or air temperature as calculated by means of equation 6. These data show the extreme slowness of temperature rise at this epidermal-dermal junction. In fact, under these conditions, the epidermal temperature even after a heat exposure of 15 minutes was far lower than the temperature of the heat source, and actually an animal would succumb to hyperthermia [1] long before the temperature of the skin approached that of the air.

Although the data for the time-temperature relationships at the skin surface are not given, they can be readily computed by putting L (the epidermal thickness) equal to zero in equation 6. If this be done, it will be found that except for the first 20 seconds of heat exposure, the

skin surface temperature was not significantly different than the values recorded in Table VI for the basal-epidermal temperature. This is due to the fact that under the conditions specified, heat transfer to the skin was the controlling factor. Thus, these data can be taken also as the temperature of the skin surface as a function of time.

A comparison of Tables V and VI indicates the importance of the mode of imparting heat to the skin surface in the epidermal time-temperature relationships. In fact, for a given source temperature, a mechanism that enables the surface temperature to be immediately brought to, and maintained at, the source temperature has, on a time basis, at least a thousand times greater propensity to injure the epidermis than a heat source which raises the skin temperature by means of radiation, and conduction and convection of relatively immobile air.[1,2,12]

TABLE VI

The Computed Time-Temperature Relationships at the Epidermal-Dermal Interface When an Entire Animal (~ 30 cm. Diameter) Is Surrounded by an Envelope of Ambient Air and Radiant Heat That Results from a Constant Temperature Source

	Circumambient temperature				
	80° C.	100° C.	125° C.	150° C.	175° C.
	Heat transfer coefficient,* H, in calories per sq. cm. per minute per °C.				
	0.015	0.019	0.021	0.024	0.026
Time	Temperature at basal epidermal layer†				
(*seconds*)					
0	35° C.	35° C.	35° C.	35° C.	35° C.
10	37	39	40.5	44	46
20				46.5	49
30	38.5	41.5	44	49	52
40				51	54.5
50	39.5	43.5	46.5	53	57
70	40	44	48	56	60
100	41	45.5	50	59	64
130	42	47	52	61	
160	42.5	48.5	54.5	63	
200	43	50	56	65	
300	45	52.5	59		
400	46	55	63		
500	47				
600	48				
800	50				
1000	50.5				
1200	51				

* In order to make these data directly comparable to the published experimental investigations,[1] the radiant contribution to H was computed by using a radiant temperature 20% in excess of the air temperature.

† Computed by means of equations 1, 2, 5, 6, 6a, 6b, and 7. Due to both the thinness of the epidermis and the slow rate of heat transport to and through the skin, there is no appreciable difference between these temperatures and those of the skin surface after the first 20 seconds of heat exposure.

V. SUMMARY

The various physical factors which determine the transfer of heat energy to and through the skin and the temperatures attained thereby have been defined and discussed. A general theory of heat flow, which

enabled the estimation of the time-temperature relationships within the epidermis during exposure to heat, was developed.

The thermal conductivities and heat capacities of epidermis, dermis, and subcutanous fat and muscle were measured *in vitro*.

Experimental observations pertaining to the rate at which thermal energy is taken up by the skin, during surface exposures of varying intensity, and the sub-surface thermal gradients established therein, have been presented.

The time-temperature relationship at the dermal-epidermal junction was computed under two greatly different experimental conditions: (i) when the skin surface temperature was immediately brought to, and maintained at, the temperature of the heat source, and (ii) when the entire skin surface was exposed to specified circumambient and circumradiant temperatures. These data indicate the extreme importance of the mode of applying heat to the skin surface in the time-temperature relationships within the epidermis.

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A HISTOLOGICAL STUDY OF SKELETAL MUSCLE IN ACUTE ISCHEMIA *

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The death of cells is a complicated process which may be assumed to proceed in an orderly manner depending upon the initiating cause.[1] In the progress of studies upon voluntary or skeletal muscle it became necessary to make correlation between cell death and its early morphological manifestation. Muscle is of exceptional value, owing to its contractility, as a mechanism in which the morphological alterations constituting necrosis may be studied conveniently. The present investigation undertakes, therefore, to define the early histopathological alterations accompanying the necrosis of ischemic voluntary muscle and to correlate these changes with the times of loss of viability and contractility in this tissue.

Earlier workers,[2-8] because of interest in the pathogenesis of Volkmann's contracture, have devoted most of their attention to the chronic lesions arising from ischemia of voluntary muscle, and have given little attention to the initial phases or changes that occur during the first 24 hours. It has been strongly indicated [7] that the changes peculiar to Volkmann's contracture commence during the first hours of ischemia and are irreversible soon thereafter; however, no record of a detailed study of that initial period has been found. Those who have studied Zenker's degeneration [9-13] have similarly paid little attention to the very early changes and applied themselves to the later manifestations of the lesion or confined their early studies to artificial systems *in vitro*. Indeed, many of these workers have indicated that vascular occlusion causes no change in voluntary muscle even after 18 hours. In studies of skeletal muscle regeneration,[12,14] the phenomena analyzed closely are mainly those that occur after a lapse of 1 or more days following injury or repair. Consequently, despite their importance, the initial lesions and their pathogenesis remain to be described.

METHODS

A state of complete, acute ischemia was induced in the hind limbs of rabbits weighing 2000 to 3500 gm. and in albino rats of the Sprague-Dawley strain with an average weight of 250 gm. In the rabbit the

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ischemia was produced in two ways: by ligation of vessels and by application of tourniquets. The animals were operated upon under amytal anesthesia, occasionally supplemented with ether; the common iliac, common femoral, the inferior epigastric arteries and conspicuous branches of the common femoral artery were doubly ligated, under sterile precautions. Ligation of these particular vessels theoretically should preclude intervention of the collateral circulation, such as was described for the hind limb of the normal rabbit,[15] and the efficacy of these ligations was empirically indicated by one observer.[7] By this method a more complete ischemia of certain muscles was obtained than by selective ligation of the arteries to the particular muscles, because even with careful occlusion of the main vessels, a muscle may retain some effective vascularization from its connection with periosteum.[16] In 12 rabbits, including a few weighing 1500 gm., a tourniquet was applied tightly around one thigh so as to occlude arterial blood flow for the desired interval; these animals were also under amytal narcosis. The duration of the experiment was measured from the initial ligation of the common iliac artery, or the application of the tourniquet, until the muscle was removed for fixation. On the other hand, in rats ischemia was induced only by application of the tourniquet to the thigh under nembutal anesthesia. The duration was limited to 12 hours or less because the animal may either mutilate the occluded limb or bite off the tourniquet.

After the required time interval the animals, with further anesthesia when necessary, were prepared for operation. The muscles of both hind limbs were exposed and tested *in situ* for contractility with a faradic current, applied directly to the muscle through metal electrodes, which derived current from a DuBois-Reymond coil, arranged for faradization by a Neef's hammer. The strength of the current was gauged so as to produce a vigorous contraction of the normal limb muscles and was then used to test the contralateral counterpart in the ischemic limb. Contractions were graded as absent, weak, or tetanic, relative to those in the normal limb.

For histological studies the tissues were placed in 10 per cent neutral formalin, absolute ethyl alcohol, and freshly prepared Zenker's solution, at 37° C. Tissues were embedded in paraffin, cut longitudinally, approximately 8 μ in thickness, and stained with hematoxylin and eosin, and Mallory's phosphotungstic acid hematoxylin. The numerical incidence of abnormal muscle fibers was calculated approximately, by a count of 100 fibers in each slide, and represented as a percentage. In all instances comparisons were made between ischemic muscle and the contralateral normal muscle of the same animal. In addition,

teased unfixed fragments of ischemic and normal muscle from rabbits and rats were examined. Several samples of human muscle, taken at necropsy within an hour of death, were also studied.

Experimental Observations

Numerous normal muscles, fixed immediately in either formalin or Zenker's fluid, afforded a picture with which the alterations in ischemic muscle might be contrasted. Certain peculiarities of normal, rapidly fixed skeletal muscle were conspicuous. Of these the most salient was the prominence of longitudinal striations, which contributed largely to the pattern of the fascicles; the fibrils composing the striations were wavy or loosely undulant, usually giving the particular group of fibers a watered-silk appearance (Figs. 1 and 2). In association with this the individual fibers were compactly arranged and so closely approximated that, except for an occasional linear arrangement of nuclei, they frequently could not be distinguished and resembled a syncytium. Because of this I have designated these compact structural forms as "syncytoid." In contrast to the prominence of the longitudinal striations and fibrils, cross striations were inconspicuous. In fact, the definition of the longitudinal and cross striations was reciprocal; the distinctness of the one was proportional to the vagueness of the other.

It may be said, therefore, that the normal mammalian skeletal muscle (rabbit, rat, man) is syncytoid, with conspicuous longitudinal striations and vague cross striations. This differs from the pattern usually regarded as normal, in which the structure of the cross striation is accentuated.

When ischemia was of sufficient duration this pattern was altered in a significant manner. The syncytoid structure was lost early, owing to a departure of the fibers from their collective compact arrangement. They showed widespread individualization and separation by clear structureless spaces (Figs. 3 and 5). The individualized fibers appeared compact and cylindrical. The longitudinal striations in such fibers were vague or absent, and when present were no longer undulant. In contrast the cross striations (Fig. 4) were very manifest and became a dominant feature. With shorter periods of ischemia the finer structure of these cross striations did not differ from that in normal muscle. Following longer periods of ischemia a new type of cross striation appeared with increasing frequency. This differed from the normal, owing to the inordinately great bulk of the anisotropic segment, which was excessively wide and broad in contrast with the narrow isotropic segments (Fig. 6). Eventually the enlarged anisotropic segments assumed the appearance of a series of adherent disks, a feature enhanced

by the splitting away or cracking off of these segments (Fig. 7). The longer the period of ischemia the more widespread and well developed were these changes, particularly those affecting the transverse striations (Fig. 8).

The degree to which changes occurred differed considerably in the various muscles of the ischemic limb (Table I). In the series of rabbits studied the proximal thigh muscles were usually least affected, whereas the flexors and extensors of the leg were always involved to a degree dependent upon the duration of the ischemia. Of the various muscles examined the tibialis anticus reflected most constantly the duration of the ischemia, whereas in the other muscles no regularly reproducible pattern of behavior was demonstrable. In view of the constancy in

TABLE I

The Percentage of Ischemic Muscles Showing Abnormal Fibers in the Rabbit Series

Muscle	No. of muscles examined	No. with abnormal fibers	No. expected with abnormal fibers	Percentage*
Gracilis	11	4	11	36
Adductor magnus	11	2	11	18
Quadriceps	10	3	10	30
Gastrocnemius	20	11	14	78
Tibialis anticus	25	19	19	100

* The percentage is based on the number of muscles involved compared with the number expected to be involved from the duration of ischemia.

gradation of response of the leg muscles, especially the tibialis anticus muscle, this muscle was selected to present the development of the morphological changes due to ischemia, although similar alterations occurred in other muscles, but less frequently.

An alteration in structure was first observed in the muscles of animals subjected to ischemia for at least 4 hours. This was characterized by individualization of fibers, regression of longitudinal striation, and enhancement of cross striation. After 6 hours of ischemia there was, in addition, a change in the type of cross striation to that of abnormal anisotropic disks as described above. The number of fibers composed of such disks increased with the duration of ischemia (Table II), until after 18 to 24 hours nearly all were affected, with considerable fragmentation and splitting off of individual anisotropic disks. The onset of depression of contractility regularly preceded the observed morphological changes to a significant extent. The contractions were vermicular and weak after 2 hours, and entirely absent after 4 hours of ischemia.

On the other hand, in the rat (Table III) these changes were first

seen at the second hour and were well developed, with disk formation by the fourth hour. Thereafter the progression toward more extensive numerical involvement was identical with that in the rabbit. The contractions were unobtainable or weak following an ischemia of 2 hours and could not be elicited after longer periods. When this absence of

TABLE II

The Time of Appearance of Individualization of Fibers and Abnormal Disks, Correlated with Disappearance of Contractility, in the Tibialis Anticus of 25 Rabbits

Duration of ischemia	No. of animals	Individualization		Abnormal anisotropic disks		Contractible muscles
		No. of animals affected	Fibers	No. of animals affected	Fibers	
(*hours*)			(*per cent*)		(*per cent*)	
2	4	0	0	0	0	4
4	4	4	60	2	10	1
6	4	4	100	4	42	0
12	4	4	100	4	60	0
18	2	2	100	2	70	0
24	4	4	100	4	90	0
48–96	3	3	100	3	90	0

contractile response was correlated with the type of lesion it was apparent that it first became absolute at about the time of, or just prior to, the appearance of the abnormal cross striations. The association was that loss of function preceded the formation of the disks, whereas the presence of disks invariably indicated a loss or depression of function dependent upon the extent of the change.

TABLE III

The Time and Extent of Occurrence of Individualization of Muscle Fibers and Abnormal Cross Striations in Ischemic Tibialis Anticus of 14 Rats

Duration of ischemia	No. of animals	Individualization of fibers	Abnormal anisotropic disks	Contractible muscles
(*hours*)				
1	3	0	0	3
2	4	4	0	2
4	3	3	3	0
8	4	4	4	0

In a series of 14 rats in which the blood supply was permitted to return to the limb after varying periods of ischemia, this relationship was apparent. The muscles deprived of blood for an interval less than 12 hours contained a few small areas of normal syncytoid tissue, commingled sparsely with the numerous abnormal fibers composed of disks. These muscles were only weakly contractile. The muscle ischemic for 12 hours never regained its function and was composed completely of abnormal fibers. It is noteworthy that even after an ischemia of 3

hours the return of blood flow did not prevent the further extensive development of abnormal fibers.

During the early stages of cytoplasmic change no alteration in the nucleus was detectable. Normally the nucleus is of a diffuse, hazy, bluish gray (hematoxylin and eosin) with a few discrete, ill defined clumps of chromatin. After continuous ischemia for 12 or more hours the haziness vanished and the chromatin clumps were more conspicuous and numerous, leaving a clear structureless nucleoplasm. After 24 hours the nuclear membrane faded until eventually it completely disappeared through karyolysis with disruption of the entire structure.

Discussion

In freshly fixed rabbit, rat, and human skeletal muscle a compactness of structure which imparted a syncytium-like appearance was observed. This structural arrangement of closely adherent fibers in skeletal muscle has led certain observers [17-19] to consider it as syncytial, like cardiac muscle tissue. However, I am not inclined to accept the reasoning that either a syncytium-like appearance or difficulty in teasing the fibers apart warrants the designation of this tissue as a true syncytium, because the ease and completeness with which the fibers separate in the earliest stage of ischemia strongly indicate that the union between adjacent fibers is not intimate nor tenacious. However, owing to its constancy in normal muscle, this syncytium-like structure may be regarded as an important histological feature of healthy muscle, which has been designated as "syncytoid." This feature, together with the scarcity and vagueness of cross striations which Leser [2] observed and the accentuation of longitudinal striations, affords a picture strikingly different from that usually drawn by histologists, who accentuate the cross striations. Schäfer [20] did remark, however, that longitudinal striation is seen better in proportion as cross striation is less marked. Millar [21] advocated that for the better demonstration of the structure of skeletal muscle it is preferable to use tissue which has been cooled overnight at ice box temperature, because otherwise the cross striations are vague and interfered with by longitudinal striation. Mallory [22] emphatically stated that to demonstrate the "myoglia fibers" autopsy tissue is practically useless and urged the use of muscle tissue obtained at operation. This inverse proportion of longitudinal and cross striations, with the enhancement of one at the expense of the other, tends to substantiate Carey's [23] concept of the entire muscle fiber as a single morphological and functional unit composed of an undifferentiated "neuromyoplasm." Certainly the reciprocal evanescence of both structures does not support either as the sole permanent structural unit.

The considerable importance attached to the mere presence of cross striations as an indication that a particular fiber or group of fibers is normal requires modification. The finding that, under the conditions of acute and chronic ischemia, the cross striations are rendered conspicuous and coarse, and are altered in type, suggests not only that cross striations do not always represent viable muscle but that they may, if of a certain type, actually signalize nonviable, degenerated fibers. This type of peculiar discoid cross striation was first described by Bowman,[24] although he believed it to be a normal feature; such cross striations have been referred to as Bowman's disks. They have been demonstrated by Leser [2] and photographed by Clark,[14] under circumstances which leave no doubt of their degenerative nature; Clark named them "conchoidal plates," and found them in degenerated muscle grafts 7 and 11 days old. Clarke [8] observed them in muscle tissue from cases of clinical and experimental Volkmann's contracture, of 70, 97, and 300 days' duration. The significance of the disks has usually been overlooked; for example, among Griffiths' [7] illustrations of ischemic contracture one shows these peculiar cross striations which he simply referred to as "preservation of striations," as have other observers including Wells.[10] In an illustration of Fishback and Fishback [9] where an absence of striations is indicated, these disks are conspicuous.

I have used the tibialis anticus to determine the time and rate of appearance of these changes. This muscle invariably underwent massive necrosis when ischemia was sufficiently prolonged. Although Wilson [6] indicated that the quadriceps was most frequently involved in his cases, he based his figures on scattered foci of necrosis and excluded muscles with extensive necrosis, so that his criteria were different. When necrosis occurred in other muscles in my series the rate of appearance and character of the lesions paralleled closely those found in the tibialis anticus.

In numerous sections of normal muscle from a variety of species, including man, rabbit, and rat, I have been unable to find Bowman's disks. On the contrary, they are a constant feature in all of my examples of *ischemic* muscle, with a constancy of time of appearance and rate of development. Since they are coincident in time of appearance with biochemical irreversibility in ischemic muscle,[25] they may represent the morphological manifestation of structural irreversibility consequent upon the establishment of static biochemical equilibrium or death due to ischemia. According to Schoenheimer,[26] in order to maintain structure against its tendency to collapse energy must be expended continuously. Under circumstances in which the oxidation, phosphorylation, and synthetic processes are known to cease, *e.g.*, ischemia,

their cessation is closely followed by such structural collapse, which is seen, in this instance, as a peculiar aggregation of the cytoplasmic constituents.

It is averred by Clark [14] that the formation of these disks is induced by formaldehyde used as a fixative, which cross links such chain molecules of myosin as may be in a more parallel arrangement. Against this it may be cited that Bowman [24] first described these structures 53 years before formaldehyde was introduced into cytological technic.[27] Furthermore, I have demonstrated them with fixatives other than formaldehyde, *e.g.*, Zenker's fluid and alcohol, and in freshly teased ischemic muscle. Moreover, Fischer [28] found that 40 per cent formaldehyde has only slight power of precipitation of proteins and a 4 per cent solution none whatever; on the contrary it renders protein solutions incoagulable by many coagulating agents. It may be pointed out in addition that if formalin alone produced this alteration it should be seen in the normal formaldehyde-fixed muscle, where it was never found. Nevertheless, it is not improbable that the fixative acts upon some fundamentally altered protein, which, since it comprises most of the muscle protein, is most likely myosin. The manner of action postulated by Gustavson [29] is that formaldehyde effects methylene cross links between peptide chains and micellar units; this view is shared by Theis and Ottens [30] and by Wilson.[31] Since this conclusion is reached from study of the collagen type of protein molecule and not the myosin-keratin type, and since the detailed structure of protein molecules is still largely unknown,[32] as is also the exact mode of union of formaldehyde with them, it would be hazardous to attribute such a change in the protein of ischemic muscle solely by analogy to altered or new chemical linkages. Furthermore, with the recognized importance of "coacervats" [33] in biological phenomena, their state in all systems reaching the equilibrium of death requires consideration. That alteration in colloidal state may constitute the basis of these changes is indicated by Bechhold,[34] who may be quoted:

> "*With the occurrence of death protoplasm gelatinizes,* Brownian movement of the smaller particles ceases, and the structure of the gel appears in the ultramicroscope as a conglomeration of many reflecting platelets. It makes a substantial difference whether the protoplasm slowly dies or is suddenly killed by a fixative (alcohol, formalin, etc.). In the first instance there is a precipitation (flocculation), whereas, in the latter there is a *stiffening;* this difference may be readily recognized under the ultramicroscope."

Whatever may be the nature of the underlying change, its importance as a morphological manifestation appears to be that it represents a terminal structural event in the process of cell death. Under restricted

conditions of ischemia it is possible to enumerate this series of events as cessation of the processes of oxidation and phosphorylation, depletion of biochemical reserves, loss of contractility, biochemical irreversibility, and finally irreversible structural change or degeneration.

Summary and Conclusions

1. Skeletal muscles of rabbits and rats, rendered ischemic for periods of from 1 to 96 hours by ligation of vessels and application of tourniquets, were studied histologically and compared with normal contralateral muscles.

2. The characteristics of normal, rapidly fixed muscles are a syncytoid structure with vague cross striations and a conspicuousness of undulant longitudinal striations. Nuclei are deeply basophilic, with a fine chromatic network.

3. With ischemia of 2 to 4 hours' duration the fibers are individualized, longitudinal striations disappear, and cross striations become a conspicuous cytological feature. After longer periods of ischemia abnormal anisotropic disks, Bowman's disks or conchoidal plates, appear and involve the muscle fibers in increasing numbers up to 18 hours of ischemia, at which time they are nearly ubiquitous. They are true degenerative forms and not artefacts caused by fixation and sectioning.

4. Weakness or absence of contractility precedes and accompanies the appearance of these disks and is correlated with their presence and extent of involvement, so that they serve as a clear indication of nonviable fibers and constitute a morphological manifestation of cell death in skeletal muscle.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 89

FIG. 1. Normal, formalin-fixed skeletal muscle of the rabbit. The fibers are closely approximated and appear syncytial. Hematoxylin and eosin stain. × 70.

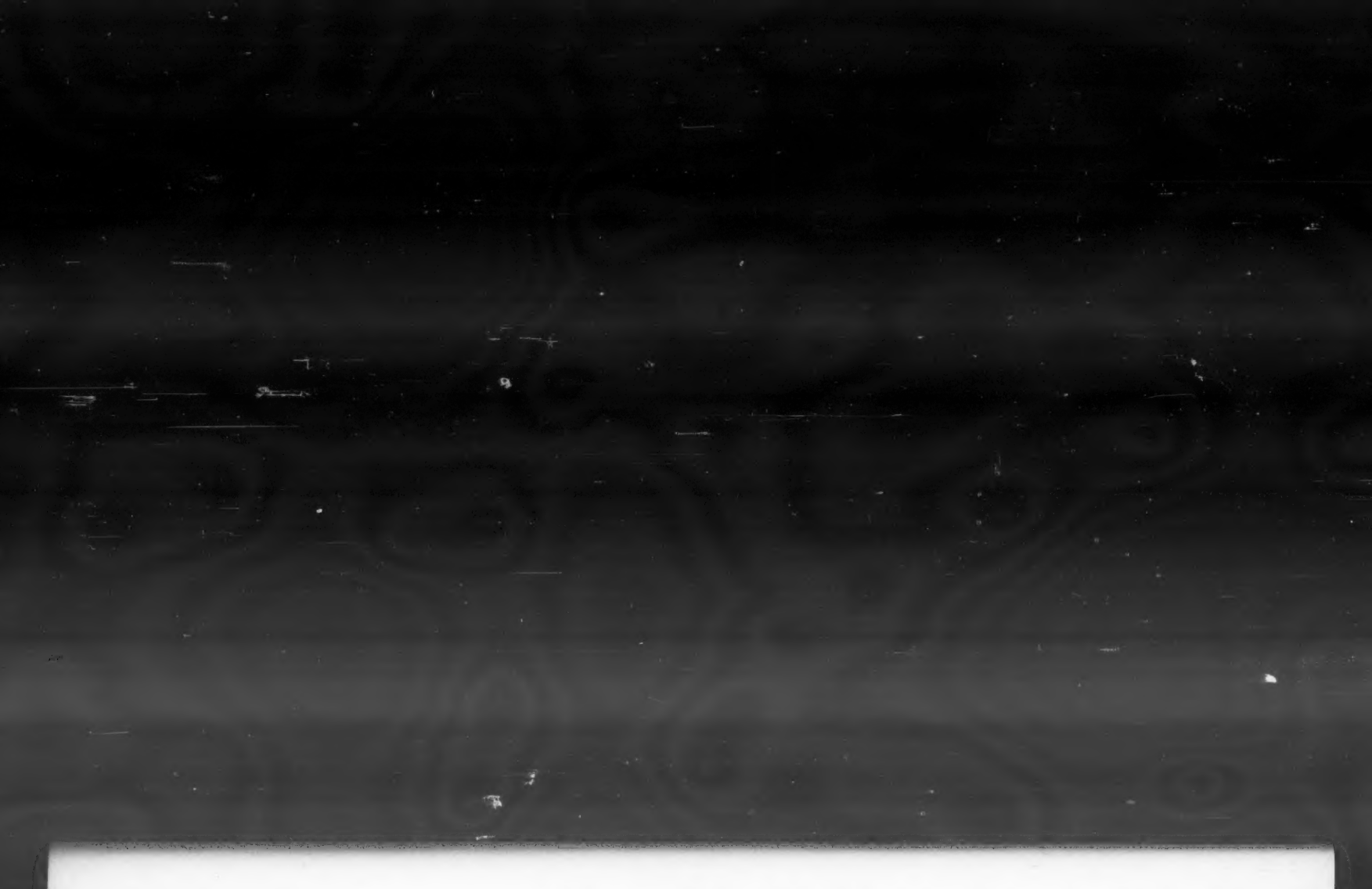
FIG. 2. Normal tibialis anticus muscle from the rabbit. The longitudinal striations are strongly evident. Cross striations are indistinct and fine. Fibers are not separate. Hematoxylin and eosin stain. × 300.

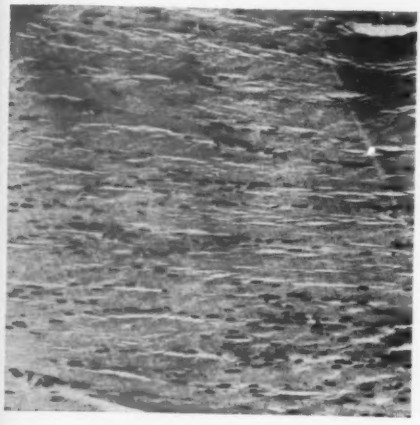
FIG. 3. Tibialis anticus of a rabbit after 4 hours of ischemia. The muscle fibers are individualized and separate. Hematoxylin and eosin stain. × 70.

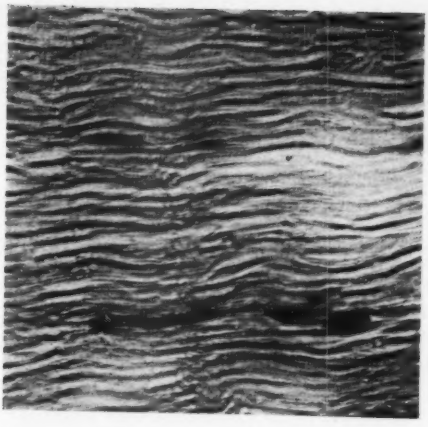
FIG. 4. High power photomicrograph of the same muscle seen in Figure 3. The longitudinal striations are not seen clearly, but the cross striations stand out prominently, with well marked isotropic bands. Hematoxylin and eosin stain. × 300.

FIG. 5. Tibialis anticus of a rabbit after 6 hours of ischemia. The individualization is further accentuated. Contours of the fibers appear more cylindrical. Hematoxylin and eosin stain. × 70.

FIG. 6. High power view of the same muscle seen in Figure 5. The new type of broad anisotropic disk and the splitting off of these disks are notable features. Hematoxylin and eosin stain. × 300.







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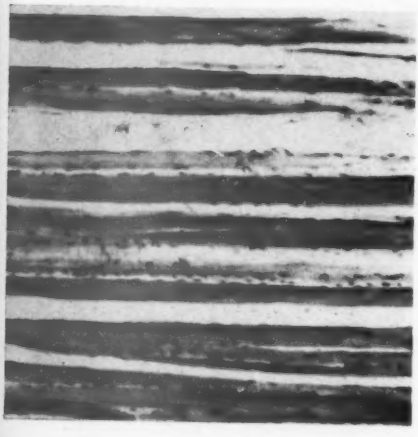

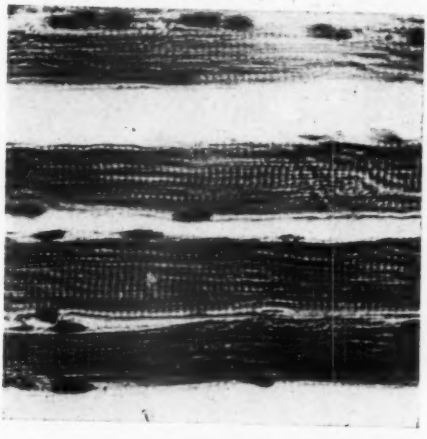

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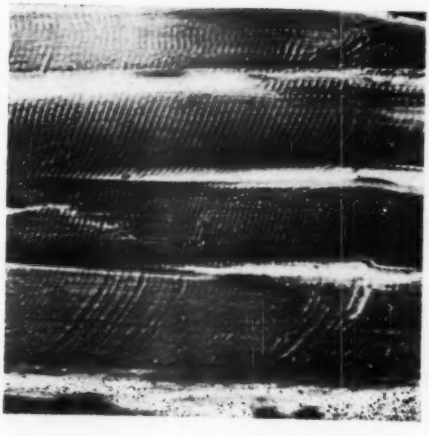


6

Harman

Skeletal Muscle in Ischemia

PLATE 90

FIG. 7. Tibialis anticus of a rabbit after 96 hours of ischemia. The fibers are broad, individualized and have developed multiple transverse cracks. Hematoxylin and eosin stain. × 70.

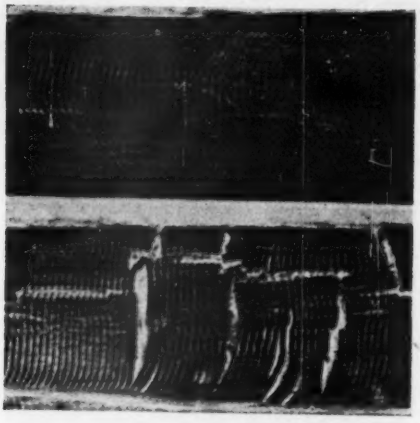
FIG. 8. High power view of the same muscle seen in Figure 7. Here the splitting off and isolation of disks, responsible for the transverse cracking, are seen. Hematoxylin and eosin stain. × 300.

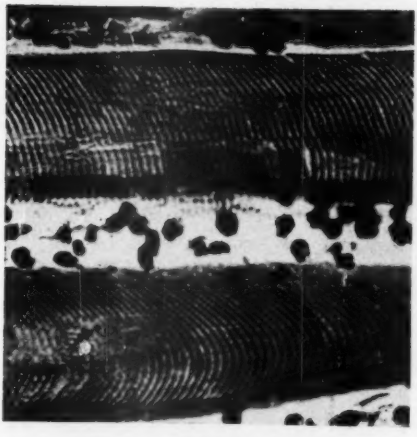
FIG. 9. Zenker-fixed tibialis anticus of a rabbit after 96 hours of ischemia. The fixative accentuates the Bowman's disks. Hematoxylin and eosin stain. × 300.

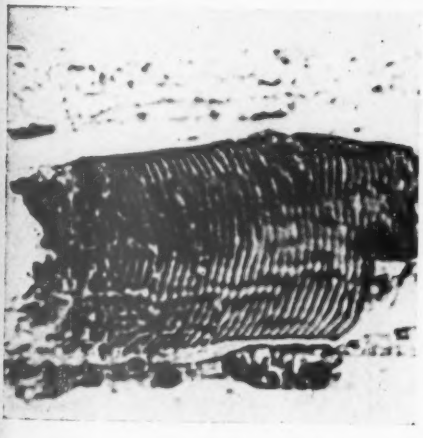
FIG. 10. Extensor muscle (tibialis anticus) of a rat after 12 hours of ischemia. The alterations are similar to those in the rabbit. Tourniquet was released shortly before the muscle was excised. Hematoxylin and eosin stain. × 300.

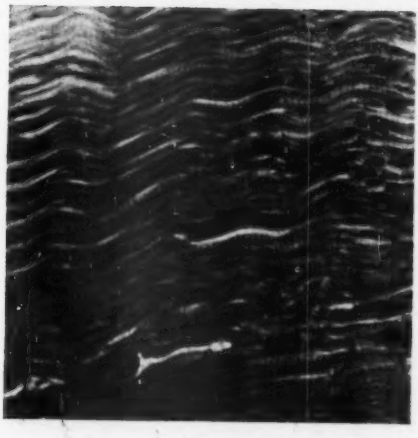
FIG. 11. Tibialis anticus of a rat after 8 hours of ischemia. The fragmentation, disk formation, and cracking are evident. Hematoxylin and eosin stain. × 300.

FIG. 12. Normal human pectoralis major muscle, taken at autopsy within an hour of death. The pattern is identical with that of normal rabbit and rat muscle. The transverse striations are more conspicuous, however, in autopsy tissue. Hematoxylin and eosin stain. × 300.









Harman

Skeletal Muscle in Ischemia

THE BENIGN GIANT CELL TUMOR OF TENDON SHEATHS

An Example of Sclerosing Hemangioma *

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The classification of a tumor is usually based upon the morphologic characteristics of its numerically predominant cell, which reveal its relationship to some normal anatomic structure. This method, simple and direct, may fail completely if the correlation it demands is lost because of changes induced in the form of the predominant cell by neoplastic change.

An example of a lesion representative of this difficulty is the benign giant cell tumor of tendon sheaths. Referred to in the literature by various descriptive terms such as giant cell tumor, xanthoma, and tendon sheath myeloma, it is characterized by the formation of a slowly growing benign tumor, the histologic make-up of which consists of large numbers of multinucleated giant cells, certain lipid-laden cells, and frequent collections of macrophages containing hemosiderin pigment. These cellular components are supported by a dense stroma of fibroblasts and collagen. Many possible relationships are suggested by these numerous, seemingly individual cell types, and when only a single specimen is available for study, attempts to weave them into a unit process fail. When a series of tumors is reviewed, certain sequential tissue changes will be observed, thus presenting an alternative method of study by which the behavior of individual tissue elements may be tabulated for integration when sufficient factors are established. This approach has been used with success by Gross and Wolbach [1] in their study of sclerosing hemangiomas.

Summary of Literature

Since the original description of the giant cell tumor by Broca in 1861 (cited by Heurtaux [2]), a considerable literature has accumulated.

Significant reviews have been published by Heurtaux,[2] Tourneux,[3] Bellamy,[4] Garrett,[5] Mason and Woolston,[6] and Galloway, Broders, and Ghormley,[7] the most comprehensive being the latter (1940), in which 339 cases were collected. In 1941, Jaffe, Lichtenstein, and Sutro [8] added 55 cases, but since then only occasional single case reports have appeared. Because of these numerous exhaustive surveys only a sum-

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mary of the significant data pertaining to the giant cell tumor will be presented.

Earlier writers, stressing cellular pleomorphism, classed the growth with sarcomas, but as the weight of clinical evidence increased its benign nature became evident, a fact first realized by Heurtaux [2] in 1891. There then followed a brilliant attempt by Bellamy [4] in 1901 to interpret the tissue antecedent to the tumor. He listed as characteristic "giant cells, proliferating endothelium, and cells showing advanced stages of fatty degeneration" and concluded that the tumor was an endothelioma of angioblastic origin. Twelve years later Fleissig,[9] comparing the lesion to a granuloma, interjected the possible inflammatory origin of the tumor, an opinion shared at present by Jaffe and coworkers [8] who regard the "phagocytic property of the stromal cell and progressive collagenization and hyalinization" as fundamental. Recently, Thannhauser and Magendantz,[10] in their excellent study of xanthomatous diseases, included the tendon sheath lesion, cataloguing it under the heading of primary essential xanthomatosis. Although only two cases of xanthoma of tendon sheaths were presented in their paper, a more intensive study of 88 synovial tumors by Galloway and co-workers [7] resulted in agreement. Thus at present there are three different interpretations of the mode of origin of the tumor existent in the literature: one considering it a benign neoplasm, another an inflammatory process, while the third holds it to be a tissue manifestation of a disturbance in lipid metabolism.

Materials and Methods

The material for this study was selected from the files of the Pathology Department of the Peter Bent Brigham Hospital and Rhode Island Hospital. One tumor obtained from the Pathology Department of the Beth Israel Hospital and two from the Lovett Fund Collection of the Department of Pathology of Harvard Medical School also were utilized. In this manner a total of 42 specimens diagnosed as benign giant cell tumor were chosen and the case records reviewed.

The tissues were fixed in Zenker's fluid or formalin, embedded in paraffin, and stained with hematoxylin and eosin, or eosin and methylene blue. On 30 of the specimens the following histologic technics were employed routinely: eosin and methylene blue, phosphotungstic acid hematoxylin, Mallory's aniline blue, Laidlaw's method for reticulum, and Turnbull's reaction for iron. Frozen sections of the available formalin-fixed material were stained with sudan IV, Nile blue sulfate, and scharlach R. Scharlach R was used in a diacetin (dihydroxyacetone) base. In a few of the specimens attempts were

made to demonstrate cholesterol in tissues by the technics of Windaus and of Golodetz and several unstained sections were examined under the polarizing microscope. Sections of one of the tumors were incinerated for ash residue.

Summary of Clinical Material

The clinical information available on these patients recapitulated that recorded in the literature. The tumors were painless growths causing no subjective symptoms, arising more frequently in women than in men. The ages of the patients in this series varied from 17 to 68 years, while the stated duration of the lesions ranged from 3 months to 15 years, averaging 3.8 years. The point of origin was usually in the tendon sheaths of the fingers and the majority of tumors were described by the surgeons as encapsulated, often mentioned as "shelling out" easily, although two of the growths were noted to be attached to the periosteum. Three of the 42 tumors of this series were recurrences.

Gross Appearance

Grossly, the benign giant cell tumor is a lobulated, seemingly encapsulated growth, seldom measuring more than 3 cm. in its greatest diameter. It has a firm, rubbery consistency and cuts with a tough resistance to the knife. The cut surface is dull yellow, streaked with gray trabeculae, and at its periphery a slender, brilliant orange-yellow line often parallels the border of the tumor.

Microscopic Description

In studying the tumors many unusual, enormously pleomorphic cell pictures were encountered which, despite the finding of transitional forms between the various cells, made interpretation difficult or impossible from the perspective of cell type alone. A clearer concept of the tumor process was gained when the behavior of the cellular elements was correlated with the stromal overgrowth that took place relatively rapidly in all of these specimens. In this manner the effect of sclerosis on the tumor cells might be observed and the resultant sequential changes in the tissue patterns better understood.

Those specimens in which little fibrosis was evident had a highly vascular structure composed of both arteriolar and capillary vessels, arborizing in the usual manner and separated from one another by a cellular tissue matrix. The blood vessels, frequently containing erythrocytes, were lined with elongated, oval, swollen, endothelial cells, the nuclei of which, when stained with hematoxylin, were pale dusty blue and contained some scattered chromatin material enclosed in a distinct

nuclear membrane. A prominent nucleolus was always present. Fibroblasts, occurring in greatest frequency about the larger vessels, gave off fibrils of collagen to intermingle with occasional smooth muscle cells, completing the formation of the blood vessel wall.

The cells of the intervascular tissue possessed nuclei strikingly similar to those of the endothelial cell. They were oval to round, slightly irregular, assumed a pale basophilic stain, and contained one or two distinct nucleoli. Mitotic figures were few. The plentiful cytoplasmic substance might be evenly basophilic at times but more often tended to contain clear vacuoles of lipid. Many of those cells, the cytoplasm of which stained with basic dyes, might be seen to be filled with fine droplets of lipid when stained with scharlach R in diacetin, although at this point it should be emphasized that no lipid was demonstrable in those regions of the tumor where active proliferation of these cells was taking place. When the fatty substance was abundant it was easily stained with sudan IV or Nile blue sulfate. The intervascular cells were separated from one another by a delicate lacework of collagen in which a fine reticulum was present.

Stromal overgrowth, beginning in multiple foci, was made manifest in its earliest phases by increased deposition of collagen and reticulum about the smaller blood vessels. These accumulations increased and coalesced, extending along the walls of capillaries, and constricting and usually obliterating vascular lumina, while fibroblasts reached through the tissue matrix to form a latticework of collagen segregating intervascular cells into irregular clusters. Eventually this process resulted in massive aggregates of hyalinized collagen in which little or no cellular detail was recognizable.

The collagen thus formed was stained less brilliantly by specific stains than the surrounding tendon sheath. Its structure might be fibrillar but more frequently had a glassy, homogeneous, hyaline appearance. In it, reticulum fibrils, as demonstrated by silver impregnation methods, were delicate and plentiful but elastic fibers were few. Micro-incineration revealed nothing characteristic in its mineral content.

The sclerosing process had a pronounced effect upon both the organoid and the cellular structure of the tumor. Endothelial cells thus encased in a firm sheath of collagen continued to proliferate as the fibrous tissue about them increased. As blood vessel lumina became occluded, the usual vascular arrangement of the tumor was disrupted, in some specimens to such an extent that poorly defined vascular channels opened through masses of intervascular cells. In such instances the cells in immediate relationship to such spaces usually contained large amounts of hemosiderin.

As the endothelial and intervascular cells were enclosed in groups by the sclerosing process, many coalesced to form multinucleated giant cells while others retained their individual character separated from one another by a delicate reticulum. The size of the latter cells tended to increase, and as their nuclear volume enlarged disproportionately their cytoplasm became relatively sparse but still might maintain some of its lipid content. Occasional mitotic figures occurred in these cells, showing that despite the regressive tendencies induced by the accumulating stroma, the process of sclerosis in itself was not sufficient to inhibit growth.

The configuration of the giant cell might vary from a simple rounded mass of cytoplasm containing a few nuclei to a branching stellate syncytium in which hundreds of nuclei were present. The nuclei were somewhat smaller, but had staining characteristics essentially similar to those of the intervascular cell. They were uniformly oval, having no special arrangement except in certain instances when the giant cell appeared to be in the process of fusing with an intervascular cell, in which case they assumed a polar position opposite to that of the single cell. Mitotic figures were seen rarely. The cytoplasm of these cells had a basophilic, smooth ground-glass appearance. Frequently this uniformity was interrupted by small, clear vacuoles of lipid, or golden brown granules of hemosiderin, and often intervascular cells were found completely engulfed in the cytoplasm of the giant cell.

Deposits of hemosiderin occurred in close correlation with the onset of the sclerosing process, in some instances so precisely as to define a focus of sclerosis as clearly as a specific collagen stain. As the fibroblastic stroma began to increase, fine granules of refractile golden brown pigment appeared in the cytoplasm of the intervascular cells and endothelial cells and, to a lesser extent, in the intercellular substance. When stained by Turnbull's method, the pigment granules stained deep blue, demonstrating their iron content.

As the continuity of the vascular bed of the tumor was interrupted, entrapped erythrocytes were engulfed by surrounding endothelial and intervascular cells. The degenerating red cells became swollen, lost their regular outline, and assumed a granular appearance as they were phagocytosed. The eventual disposition of the pigment probably proceeded in its usual manner, by hemoglobin breaking down to hematoidin and the latter going into solution in tissues, as none of the specimens examined showed any tendency to maintain collections of hemosiderin once the sclerosing process was well advanced.

The tumor grew in a lobular manner, dissecting through the tendon sheath and breaking it up into slender septa that penetrated varying distances into the tumor, often so far as to divide one lobule from

another. Although overwhelmed by sclerosis in its central portion, a lobule might exhibit evidence of growth at its periphery. In some specimens numerous mitotic figures might be seen in the intervascular cells as they proliferated in broad masses, pushing into the surrounding tendon sheath. Capillaries invaded the tendon sheath directly, only to become rapidly obliterated, leaving linear seams of hemosiderin and/or lipid-containing endothelial cells that continued to grow after their vascular structure had been destroyed. In consequence of this activity dense aggregates of hemosiderin appeared at the surface of the tumor and in the surrounding tendon sheath.

Although these deposits were rather sparse in 12 specimens, they were present in all of the 42 tumors examined.

When the lipophagic activity of the intervascular cell became extreme, the cell assumed a characteristic appearance to which the terms foam cell and xanthoma cell have been applied. In 22 of these tumors this tendency was not pronounced, but in the other specimens "xanthoma cells" were arranged in collar-like sheets at the far periphery of the tumor and rather abruptly differentiated from it. Less frequently they were scattered diffusely through its substance. When this arrangement was seen it was noted that the sclerosing process had failed to obliterate vascular lumina even though many of the blood vessel walls were composed of dense hyaline collagen. Neither was there any evidence of destruction of the vascular network in the solid masses of foam cells.

As seen in hematoxylin and eosin preparations, the xanthoma cell possessed an abundant foamy cytoplasm composed of small, evenly sized, clear vacuoles separated from one another by a delicate pale basophilic, granular lacework. When treated with sudan IV, Nile blue sulfate, or scharlach R, this cytoplasmic material colored brilliantly, demonstrating its lipid nature. The reticular background appeared pale blue when stained by Mallory's aniline blue method for collagen.

Chemical analyses of these tumors [7,10] showed some of them to contain large amounts of cholesterol. Utilization of the available histochemical methods for its demonstration, such as the digitonin reaction and the procedures of Liebermann-Burchardt and of Golodetz, yielded inconstant results. However, the Golodetz test was often positive, staining the foam cell a pale brown. When examined polariscopically the lipid appeared in the form of needle-like crystals, but lost its refractile qualities when the slide was warmed gently. As the tissue cooled after such treatment, many doubly refractile droplets appeared, suggesting the presence of cholesterol esters. It is thought that the needle-like forms observed represent crystalline fatty acid.

Although these lipophagic cells may be multinucleated, this feature was not pronounced as they seldom contained more than two or three nuclei. More characteristic of their behavior was the tendency of the nuclear chromatin to be condensed and the nuclear membrane wrinkled, so that the nucleus became small, shrunken, and pyknotic, lying in a large mass of foamy lipid, eventually fragmenting and disappearing completely. In such instances focal collections of lipid might remain in the tissue in an extracellular position. The lipophagic activity of the intervascular cell, manifest shortly after cell proliferation ceased, remained as a continuous function impaired only by stromal overgrowth until the death of the cell.

Although the precise nature and origin of the lipid is not understood, it is apparently derived from the blood stream and consists of a mixture of cholesterol esters and neutral fats. These substances are definitely associated with degenerative phenomena exhibited by the intervascular cell.

Discussion

As the giant cell tumor grows, it infiltrates the surrounding tendon sheath from which its capsular structure is derived. Transitional forms between the giant cell, intervascular cell, and foam cell indicate them to be morphologic variations of the same type cell, the cytologic characteristics of which are closely correlated to the stromal reaction. As stromal overgrowth proceeds and the vascular networks of the tumor are interrupted, the intervascular and endothelial cells phagocytose hemosiderin liberated from fragmented erythrocytes. Many of the tumor cells coalesce to form multinucleate giant cells. If blood vessel lumina are not obliterated by sclerosis, the intervascular cells accumulate lipid until the amounts of fatty substance become so great that the cells fragment and die. The proliferation of tumor cells is continuous, limited only by their own degenerative tendencies. Even in specimens in which sclerosis is far advanced, occasional mitotic figures occur in nests of tumor cells segregated by massive amounts of collagen. These lesions, enlarging progressively, restrained only by their own growth characteristics and characterized by a type cell occupying a definite and predictable relationship to its stroma, can be considered only as neoplasms.

The extreme vascularity of specimens uninvolved by sclerosis immediately suggests their angiomatous nature. This detail is especially well demonstrated in sections impregnated with silver by Laidlaw's method and counterstained with van Gieson's connective tissue stain as advised by Stout.[11] Proliferating sheets of intervascular cells and infiltrating capillaries seen at the surface of the tumor are integral fea-

tures of its growth, while the lipid accumulations, stromal overgrowth, and hemosiderin deposition are phases of a regressive process. These tissue reactions are manifestations of the activity of various components of the vascular system and are centered about the blood vessel pattern of the tumor. The behavior of the tumor cells, as well as the anatomic structure of specimens, uninvolved by sclerosis, define the tumor as a sclerosing hemangioma.

Opinion regarding this process as a metabolic disturbance was not confirmed by this study. Although the foam cell has been thought to be the anatomic expression of a disturbed blood lipid stoichiometry, fat, appearing in these cells secondarily, represents a degenerative phenomenon that is seen in numerous other neoplastic and inflammatory diseases.[12] Proliferation of tumor cells occurs before lipophagocytosis is demonstrable and ceases as large amounts of lipid appear in the cytoplasm of the intervascular cell.

It should be mentioned that the sclerosing hemangiomas are to be separated from those infiltrates which are definitely a part of the metabolic disturbances. Such lesions have a familial incidence and often appear in younger persons. They have been described [10,12] involving multiple tendon structures and other organs. Further investigation of this problem is warranted as the histologic description of certain of these specimens [7,10] does not coincide precisely with that of the sclerosing hemangioma.

Some authors, emphasizing the continuous increase of fibrous tissue, the phagocytic properties of the intervascular cell, and the giant cells seen in these lesions, have presumed them to be inflammatory in origin. If progressive fibrosis is to be considered a phase of an inflammatory reaction, its mechanism should be explained in a study of the stromal tissue sequences; but fibroblastic activity in these tumors is continuous, related to other cellular elements only through the effects it produces. Neither is it permissible to credit the phagocytic activity of the intervascular cell as indicative of inflammation. These items taken separately merely catalogue various cell functions but do not describe any process, as they do not integrate into a common pattern.

This tumor is believed to be the tendon sheath equivalent of the cutaneous sclerosing hemangioma described by Wolbach [13] in 1913 and more fully by Gross and Wolbach.[1] Even though minor differences between the two exist, such as a more uniform tendency toward phagocytosis of lipids and toward giant cell formation in the tendon sheath lesion, it must be remembered that these are quantitative differences, whereas the tissue sequences so clearly traced by Wolbach in cutaneous tumors are identical in both neoplasms. The sclerosing hemangiomas

of the central nervous system reported by Bailey and Ford [14] in 1942 are also identified with this group by those authors. Here again, quantitative differences are evident while tissue sequences remain the same. The stromal overgrowth occurring in tumors of the central nervous system is peculiar in that both glial and fibroblastic elements participate, but stromal behavior as well as the tumor cell components follow the patterns observed in sclerosing hemangiomas elsewhere.

Although a precise explanation of the mechanism of sclerosis was not found, it was noted in specimens of 1 year or less in duration that only minimal amounts of stromal overgrowth had occurred, an observation in conformity with another in the literature.[8] Neither was sclerosis pronounced in the peripheral portions of certain tumors in which growth, as evidenced by frequent mitotic figures in the intervascular cells, was rapid. Stromal overgrowth could not be correlated with the extent of degeneration of the intervascular cells but rather followed a slowly progressive course, as if representing an imbalance in the growth relationship between tumor cells and stroma.

The tendency of the tumor to recur, a feature hardly to be considered exemplary of a completely benign, slowly growing neoplasm, is well recognized [7,8] and three such instances are recorded in this series. Some explanation of this may be found when the tumor-tendon sheath interface is studied. Here it is seen that the portion of the tendon sheath forming the tumor capsule may be infiltrated to a considerable extent with capillaries and cell masses reaching from the surface of the neoplasm. Even though the main body of the tumor may shell out easily, fragments of actively growing neoplastic tissue may be left behind. For this reason care should be taken to extirpate the lesion widely.

The giant cell tumors occurring in the synovial structures of joint cavities and often discussed with the tendon sheath lesion as its synovial equivalent [7,8] are not included in this series. Although it is believed that such an identity exists, it is felt that the clinical and pathologic characteristics of the synovial lesion are sufficiently different to warrant separate discussion.

Conclusion

In its earliest phases of growth the benign giant cell tumor has a highly vascular structure that becomes disrupted by sclerosis, but proliferation of the intervascular and endothelial cells of the tumor continues, limited only by the degenerative tendencies inherent in these cells and to some extent by stromal overgrowth. These are features of autonomous growth and definitive of a true neoplasm.

As the development of the tumor progresses, certain of its cellular elements phagocytose lipid and hemosiderin while some coalesce with one another to form multinucleated giant cells. These activities represent behavior characteristics of the reticulo-endothelial system, and since they are centered about blood vessels it is concluded that the tumor is a sclerosing hemangioma.

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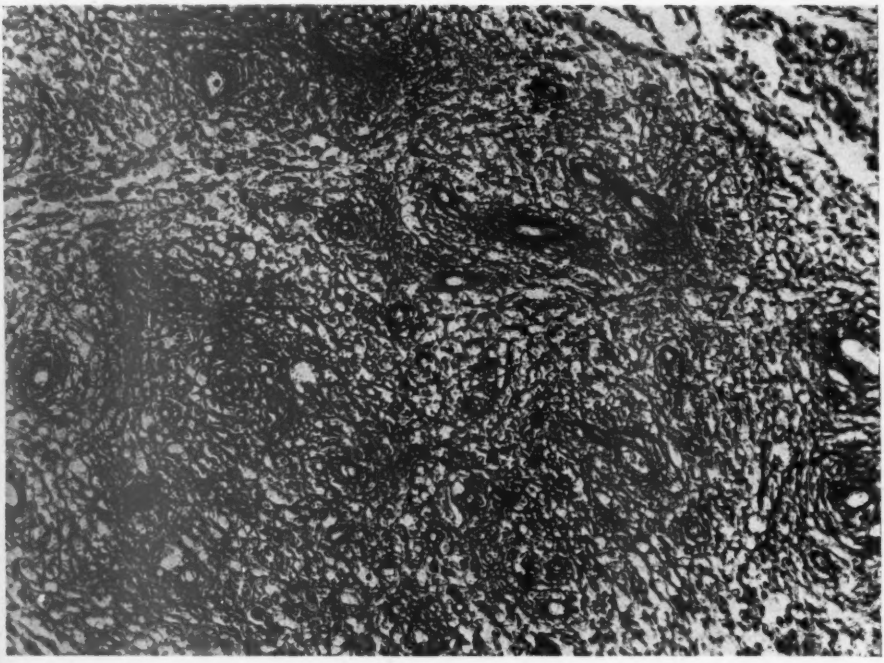
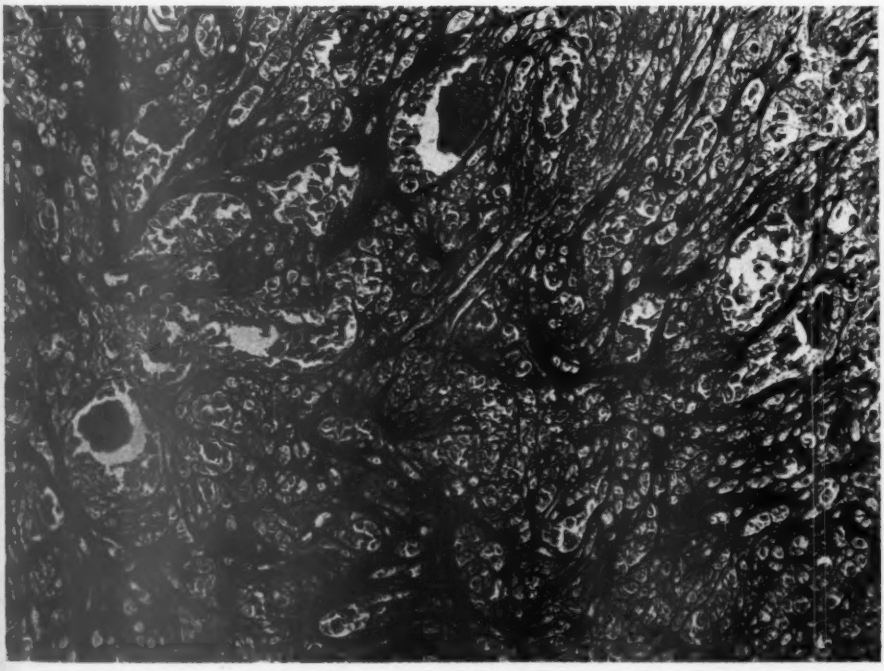
DESCRIPTION OF PLATES

PLATE 91

FIG. 1. Those specimens in which stromal overgrowth has not occurred have a highly vascular structure. Beginning perivascular accumulations of reticulum are seen. Laidlaw's method for reticulum. Van Gieson's counterstain. × 150.

FIG. 2. As sclerosis proceeds, the vascular structure becomes disrupted. Some increase in the size of the intervascular cells occurs. Laidlaw's method for reticulum. Van Gieson's counterstain. × 150.



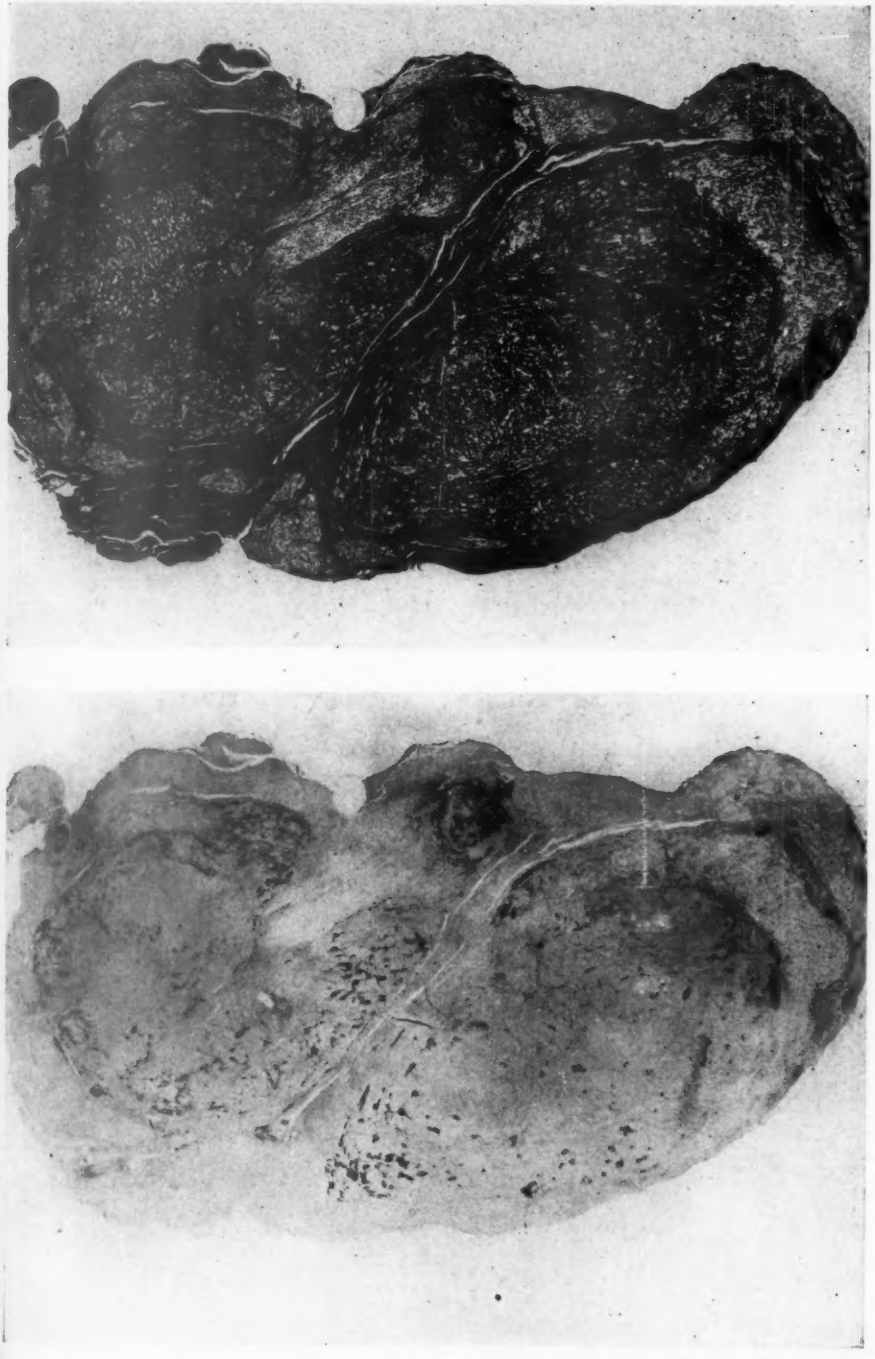


Foster Giant Cell Tumor of Tendon Sheaths

PLATE 92

FIG. 3. Stromal overgrowth involving the central portions of two tumor lobules. A rounded solitary focus of sclerosis is present in the middle portion of the upper part of the section. Laidlaw's method for reticulum. Van Gieson's counterstain. × 10.

FIG. 4. Sequential section from the tumor illustrated in Figure 3 stained by Turnbull's reaction for iron. The solitary focus of sclerosis is especially well demarcated by the dark granules of iron pigment. × 10.



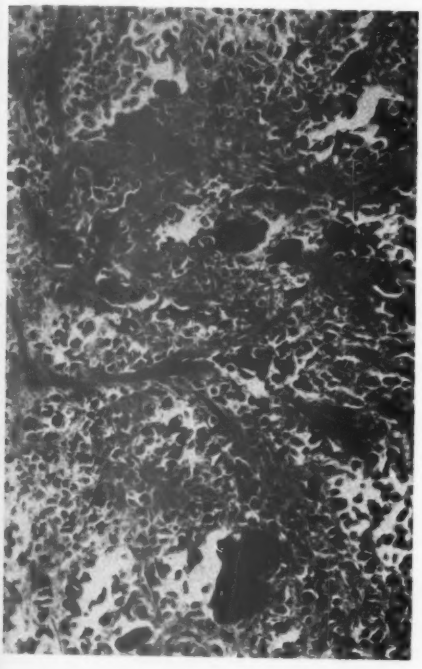
Foster Giant Cell Tumor of Tendon Sheaths

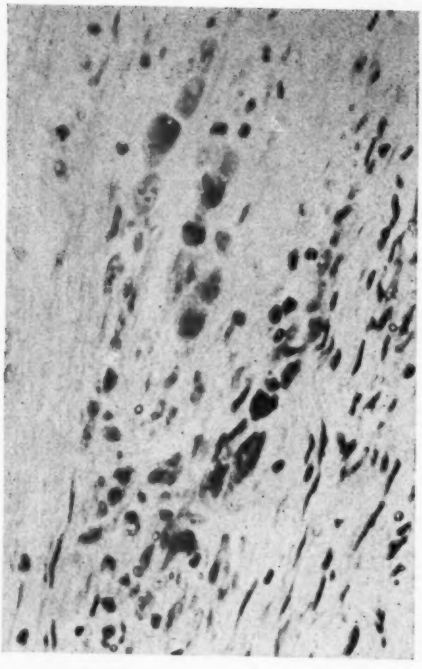
PLATE 93

FIG. 5. As the collagenous stroma accumulates, intervascular cells coalesce to form multinucleated giant cells. Recently engulfed cells are plainly visible, represented by a nucleus surrounded by a clear ring of cytoplasm. Phosphotungstic acid and hematoxylin stain. × 150.

FIG. 6. A mitotic figure in a cord of endothelial cells. There are numerous granules of hemosiderin present. Eosin and methylene blue stain. × 500.

FIG. 7. The cellularity of the tumor becomes reduced by heavy deposits of collagen. Phosphotungstic acid and hematoxylin stain. × 300.





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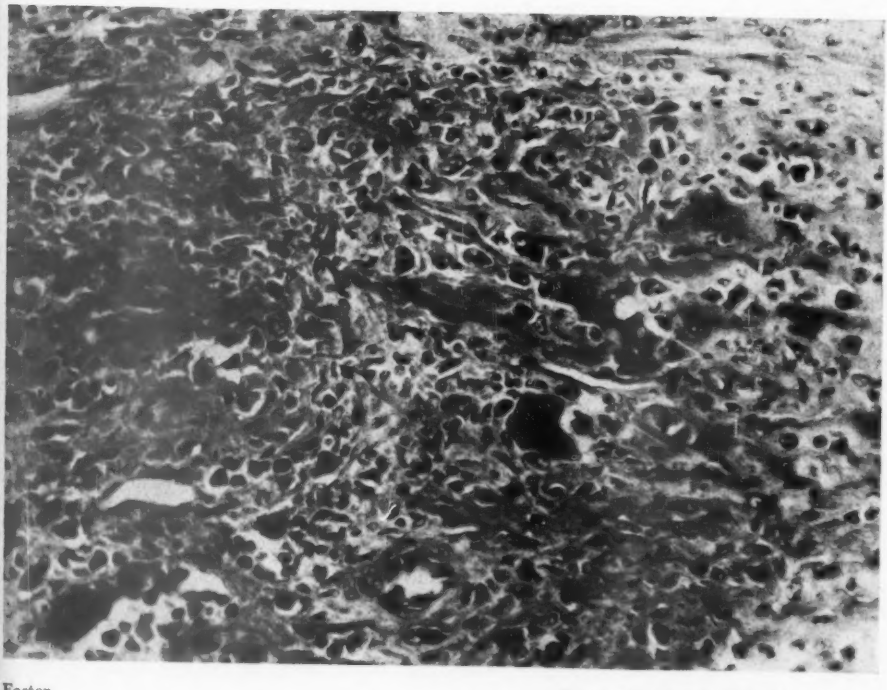


Foster

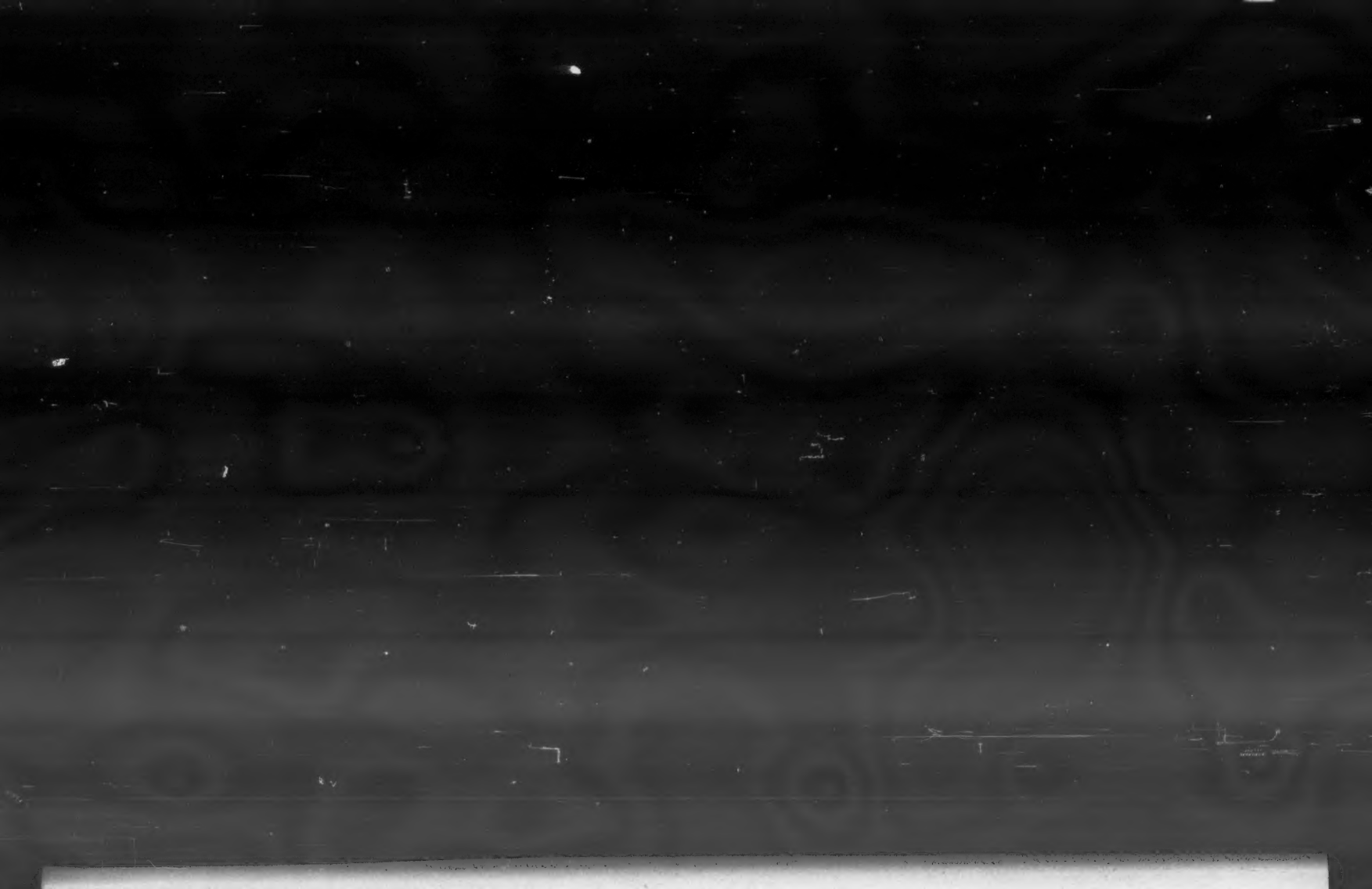
Giant Cell Tumor of Tendon Sheaths

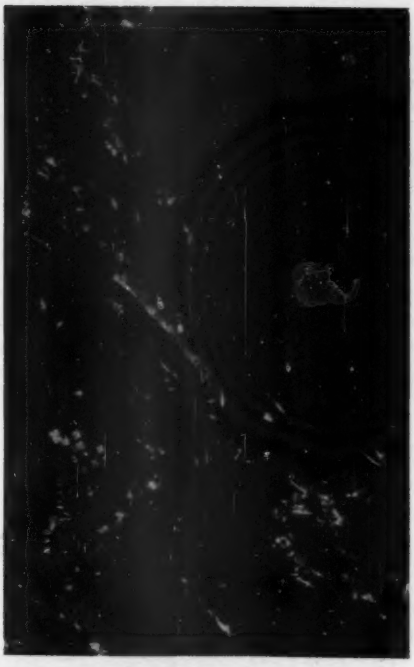
PLATE 94

FIG. 8. A mitotic figure in a nest of tumor cells segregated by the sclerosing process. Eosin and methylene blue stain. × 900.

FIG. 9. Birefringent crystals and globules of lipid seen under the polarizing microscope. The globules show the characteristic Maltese cross formation. × 150.

FIG. 10. Foam cells scattered through the tumor substance. In such instances continuity of the vascular bed of the tumor is left intact despite the heavy collars of collagen and reticulum surrounding the blood vessels. Mallory's aniline blue stain. × 150.





Foster Giant Cell Tumor of Tendon Sheaths

ENDOTHELIAL-CELL SARCOMA OF LIVER FOLLOWING THOROTRAST INJECTIONS *

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As a pathological entity, a primary hemorrhagic endothelial-cell sarcoma of the liver is an unusual and interesting finding. When it is found in a patient who has received thorotrast, its presence may be of much greater significance. Thorotrast is colloidal thorium dioxide. It was first introduced into the practice of medicine 18 years ago when Blühbaum, Frik, and Kalkbrenner [1] described its use in roentgen visualization. As a diagnostic aid it has become widely adopted. It was first used in the field of gastroenterology, later in urology, and most recently in neurology. Its use has been condemned by some and extolled by others. Reeves and Stuck [2] have pointed out its disadvantages, and Yater [3] and McClure, Jankelson, and Osgood,[4] as recently as 1944, have championed its advantages.

REPORT OF CASE

The patient was a female who, at 40 years of age, had had a cholecystectomy. Ten years later she had experienced, over a 2-week period, attacks of paroxysmal pain under the costal margin. There was a history of considerable but vague abdominal distress during the subsequent 8 years. At the end of this period, when the patient was 58 years of age, a mass was described as filling the entire right half of the abdomen. A barium enema revealed displacement of the hepatic flexure and ascending colon to the left by a large mass in the right side of the abdomen. By pneumoperitoneum there were visualized one shadow filling the right half of the abdomen, interpreted as that of the liver, and a second shadow beneath the right diaphragm of undetermined origin. Repeated serological tests for syphilis at that time were strongly positive. Because of the uncertainty of the diagnosis a commercial preparation of thorotrast was given intravenously. Three daily doses of 25 cc. each were injected and very satisfactory contrast films were obtained. The liver was obviously rotated downward. The left lobe lay beneath the dome of the diaphragm and the right lobe filled the right half of the abdomen. The thorotrast also clearly revealed a sharply defined filling defect, measuring approximately 8 cm. in diameter. This lay in the right lobe. A diagnosis of gummatous syphilis of the liver was made and specific therapy was instituted. Following adequate intramuscular and intravenous antiluetic treatment, the patient became entirely free of symptoms. Subsequent roentgenograms taken over a 6-year period clearly demonstrated a progressive diminution in size of the liver and ultimate disappearance of the gumma. Ten years after the injection of thorotrast, the patient, then 68 years of age, was described as being in good health and very active physically and mentally. It is of interest that her blood pressure was subnormal for her age, averaging about 110/60 mm. Hg. Two years later she was admitted to the hospital as an emergency case. She had suddenly experienced weakness and pallor. On admission she was restless and her pulse was rapid. Laboratory studies revealed a hemoglobin of only 37 per cent and a red blood cell count of 1,760,000. Her condition

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was very poor. Blood pressure recordings were unobtainable. Treatment with transfusions was of no avail. She died a few hours after entrance with the clinical diagnosis of "shock, the result of internal hemorrhage."

AUTOPSY FINDINGS

At autopsy the body was found to be moderately well nourished and well preserved for 70 years of age. The skin and underlying tissues were pale and bloodless. The peritoneal cavity contained 2000 cc. of fluid and freshly coagulating blood. The *liver* was rotated and displaced to the right, and lay in an almost vertical plane. It was smaller than is normal and weighed only 1050 gm. It was composed of small and large lobes separated by deep and shallow furrows. It presented the picture of hepar lobatum. It was firm, reddish brown, and the surfaces of the individual lobes were smooth. In addition to this displacement and deformity there were three other unusual findings. First, the entire capsular surface showed a fine yellowish white, thread-like marking. Secondly, the right lobe was spotted with brilliant patches of red varying from 0.1 to 2 cm. in diameter. Thirdly, there was a ragged, friable, soft, spongy mass, 5 cm. in diameter, saturated with blood, that merged with the inferior surface of the liver. This was the source of the fatal hemorrhage and this was a primary tumor. Sections of the liver revealed that this soft blood-soaked mass of neoplastic tissue was embedded in its substance, that the many small hemorrhagic areas visible at the surface were equally numerous throughout the entire right lobe of the liver (these proved to be metastases), and that the yellowish white thready lines seen through the capsule traversed the whole liver. Adjacent to the hemorrhagic tumor these lines became broader and more numerous and converged on a solitary, firm, creamy yellow, gritty mass, 2 cm. in diameter. The *spleen,* which weighed 100 gm., was soft, reddish brown, and almost bloodless. Like the liver it showed, both on the surface and on section, innumerable delicate, yellowish white, interlacing thread-like lines throughout the parenchyma. *Lymph nodes* from the hilus of the liver, mesentery, and para-aortal area contained a yellowish white substance. The *bones* were fragile and could be cut easily with a knife. The *marrow* of the ribs, sternum, and vertebrae was red. The *heart* was small and moderately firm. The *lungs* showed one interesting finding grossly. There were several small, solitary, discrete hemorrhages in the right lower lobe which proved to be metastases.

Microscopical Examination

Liver. Before enumerating or describing the very extensive and unusual pathological changes within the liver, it is important to point out that a considerable portion was still well preserved. Three significant

histological lesions were demonstrable: (1) A primary malignant tumor with metastases, (2) a very heavy deposition of thorotrast with severe damage to the liver, and (3) scars of healed syphilitic hepatitis with gumma formation.

The most important of these three findings was the tumor with its regional metastases throughout the liver. There were several noteworthy features of this tumor (Fig. 1). It had originated in an area immediately bordering the largest concentration of thorotrast in the body. It was composed of a single type of cell resembling most closely the Kupffer cell. The cells varied considerably in size; the average was about as large as a liver cell. They varied in shape. Some were nearly round, others were elongated and narrow, still others were very irregular and showed grotesque vacuolated forms with large and small, clear, blistering projections of their cytoplasm. The average cell had very little cytoplasm and this stained mildly basophilic. The texture of the cytoplasm varied from ground glass to finely granular. The outer limits of the cells were poorly defined; often no cell membrane was demonstrable. The nucleus occupied most of the cell and its shape could be compared to that of a potato. The nuclear membrane was sharp, thin, and wire-like. The nucleus stained very lightly except for two or three coarse clumps of chromatin that were suspended in a very delicate web of reticulum. Mitotic figures were rarely seen and these were atypical. Some tumor cells showed degeneration, necrosis, disintegration, and lysis. Karyorrhexis with nuclear fragmentation was a conspicuous feature of this regressive change. Other tumor cells contained inclusions consisting of thorotrast, red blood cells, leukocytes, and hemosiderin. Tumor cells containing thorotrast seemed particularly susceptible to necrosis. While tumor cells appeared singly, they tended to arrange themselves in three common patterns, namely: (a) in clusters so close together that individual cell outlines could not be identified, (b) as walls of irregular blood sinuses, bearing the same relation to liver cells as Kupffer cells elsewhere, and (c) as walls of blood vessels replacing normal endothelium. Everywhere they showed a tendency to cling to pre-formed surfaces, whether cells, basement membranes, or reticulum. They invaded, destroyed by lysis, and crudely replaced normal sinus endothelium (Fig. 2). They invaded the trabeculae of liver cells, broke up lobules, and destroyed normal patterns (Fig. 3). Their most conspicuous characteristic was to break normal endothelial barriers and produce hemorrhages which showed no sign of coagulation (Fig. 4). They grew within the lumina of branches of the portal vein and metastasized freely throughout the liver (Fig. 5). The effect of the tumor in the liver was fourfold (Fig. 6). First, its very presence destroyed

liver tissue; secondly, the hemorrhages led to compression and atrophy of liver cells; thirdly, by interfering with local circulation there was infarction and necrosis; and lastly, by destruction and rupture of the capsule of the liver there was a fatal intra-abdominal hemorrhage.

The second significant finding was the extensive deposition of thorotrast. No portion of the liver was entirely free of it, but in some areas there was so little that the normal structure was well preserved. In other fields there was so much of this material that all structural detail was entirely lost and in such areas no living or necrotic cells were recognizable (Fig.7). The thorotrast appeared as tiny granules and globules closely packed together. It was rather colorless but at times acquired a faint eosinophilic tint. As the index of refraction was quite different from that of the tissues, it could be visualized very clearly in the tissues by controlling the amount of light. Much of this substance lay quite free in the tissues; the rest was taken up by histiocytes, Kupffer cells, and tumor cells. Some cells contained little, others were so distended with it that only the cell outline was recognizable. In fields where the cells were packed, it was impossible to distinguish one cell from another. Cells distended with this material died and their contents spilled into the surrounding space. The greatest concentration was in areas of old inflammatory scar tissue. Next in degree of concentration was the region about the central vein where it replaced the liver cells of the central zone, and at times of the midzones of the lobule (Fig. 8). In this area there was some condensation and hyalinization of the stroma, but there was no significant increase in fibrous tissue. A third site was in the capsule and portal connective tissue, but though the amounts here varied considerably, they were not to be compared with the larger quantities in the scars and central zones (Fig. 9). In many of the portal areas thorotrast was deposited in histiocytes or was free. In either case it lay embedded in an excess of dense, homogeneous, hyaline connective tissue. This increase in portal connective tissue was associated with an obliteration of lymphatics and a narrowing or occlusion of veins. Occasionally in such areas a vein was partially or completely occluded by a thrombus. The increase in collagen in association with thorotrast was much greater in the capsule and portal areas than in the central zones. Liver cells bordering collections of thorotrast showed several forms of degeneration, disintegration, and necrosis. One of these was characterized by the accumulation of lipids and hyalin in the cells (Fig. 10).

The third important finding in the liver was the presence of large and small, old healed inflammatory scars. These scars accounted for the gross nodular deformity. Some scars contained little thorotrast,

others were saturated with it. There was nothing in any of the sections to suggest an active syphilitic infection.

Spleen. There was a heavy deposition of thorotrast in fibrous tissue outside the splenic capsule, in the capsule, in and about the trabeculae, in the pulp, and in the follicles, but the greatest concentration was centered about the small vessels (Fig. 11). No part of the spleen was spared. As in the liver, thorotrast tended to collect in tracts and clusters. Sometimes it was confined to histiocytes, but often, and especially in fibrous tissue, it lay quite free. The detailed structure of the pulp was poorly defined, but almost everywhere there was a relative increase in supporting collagen. This was especially true of capsule and trabeculae. The follicles were small and relatively numerous. Each showed a small central core of primitive cells bordered by a loose ring of lymphocytes. Some of the sinuses contained developing blood cells, and in one or two areas in the pulp there were found clusters of primitive erythroblasts. There was a moderate deposition of iron pigment, and at times histiocytes contained both thorotrast and hemosiderin. An interesting observation was the complete absence of polymorphonuclear leukocytes in all sections.

Lymph Nodes. Thorotrast was deposited all through the nodes, but there was the same tendency here as in the spleen and liver for it to be concentrated in masses (Fig. 12). Thorotrast was found in circulating monocytes within the peripheral and traversing sinuses. Almost as conspicuous as the thorotrast was the diminution in the total number of lymphocytes (Fig. 13). The lymphoid tissue was confined to small scattered nodules embedded in a web of reticulum. There was some bleeding into the node and, although there was a moderate reticulum and endothelial cell hyperplasia, there was no suggestion of connective tissue proliferation or fibrosis.

Bone Marrow. Solitary histiocytes and large and small nests of histiocytes laden with thorotrast were scattered throughout the marrow (Fig. 14). There was a very uneven distribution and a very definite diminution in hematopoietic tissue. There was too much fat in the marrow, even for this age. The capillaries and sinuses were numerous and dilated, and capillary bleeding was common. Erythropoiesis was abnormal. There were small nests of erythroblasts and megaloblasts free of hemoglobin. There also were clusters of normoblasts, but many of these showed little tendency to exhibit normal maturation and some appeared to be undergoing disintegration. In many fields there were no myeloblasts, no myelocytes, and no cells of the myelocytic series. There were very few mature polymorphonuclear leukocytes, and eosinophils were entirely lacking. The number of megakaryocytes was

diminished, and of those present the majority were quite immature. There were loosely arranged nests of lymphocytes replacing normal marrow (Fig. 15). The over-all picture was one of too few cells, and of these, many were very primitive, many lacked evidence of normal maturation, and many, especially of the red cell series, showed premature death. There was no hemosiderosis and no erythrophagocytosis.

Bones. There was generalized osteoporosis and in many areas trabeculae had entirely disappeared (Fig. 17). The trabeculae were thin and delicate, and some showed patches from which all bone cells had disappeared. Their margins were ragged and rough, and the ground substance showed a linear marking with a tendency to split longitudinally and even to break up. Histiocytes laden with thorotrast often lay adjacent to bone, sometimes along the surface of trabeculae, at other times about capillaries along the haversian canals. It should be pointed out that no thorotrast was found within the ground substance of bone.

Adrenal Glands. There was little thorotrast in the adrenal medulla and in the endothelial cells of the sinuses throughout the cortex. Islands of medullary tissue were replaced by nests of lymphocytes and plasma cells, and by histiocytes laden with thorotrast (Fig. 16). There was degeneration, atrophy, and fibrosis of portions of the zona glomerulosa containing thorotrast (Fig. 18).

Kidney. The normal structural detail of the kidney was well preseved, but there were two changes worthy of description. In blood vessels (Fig. 19) there was a disappearance of stained cytoplasm from the smooth muscle fibers of the walls; the elastica was interrupted and and in places absent, and the lumina were abnormally wide. The second change was that an occasional glomerulus showed a deposition of thorotrast associated with focal collapse and hyalinization of the glomerular tuft.

Lungs. In sections of the lungs taken through areas of hemorrhage there were tumor cells lining alveolar spaces distended with blood. There was a disappearance of smooth muscle from the walls of blood vessels (Fig. 20) and bronchi, and a replacement with hyaline tissue. No thorotrast was found in sections taken from the periphery of the lung.

Heart. Beneath the endocardium the muscle fibers and their cross striations were well preserved but elsewhere the fibers were swollen and their cross striations were either lost completely or were cloudy and barely discernible. No thorotrast was found, but there was a light collection of lymphocytes and plasma cells about some of the small vessels in the interstitial tissue. Small arteries were dilated and their walls were hyalinized and devoid of smooth muscle.

Final Diagnoses

Fatal intra-abdominal hemorrhage, from an endothelial-cell sarcoma of the liver showing multiple regional metastases. Thorotrast deposition and irradiation, with degeneration of liver, spleen, lymph nodes, bone marrow, bones, adrenals, kidney, and blood vessel walls. Old healed syphilitic hepatitis (hepar lobatum).

Discussion

All four significant lesions in this patient—the fatal hemorrhage, the malignant tumor, the thorotrast deposition, and syphilis—were centered in the liver. The hemorrhage was very large and sudden, and the tumor was its obvious source. This was filled with blood even after its outer surface, which was formed by the liver capsule, had ruptured into the peritoneum.

A hemorrhagic sarcoma apparently having origin in sinus endothelium is a very rare primary tumor of the liver. In this tumor the cells resembled Kupffer cells in both structure and function. They had the property of phagocytosis and could build crudely constructed sinuses. They tended to retain the same intimate association with liver cells as is shown by normal sinus endothelium. Their most striking characteristic was to destroy normal endothelium and produce multiple hemorrhages.

The significance of the thorotrast in the liver must not be underestimated. Most important was the fact that the tumor had originated in immediate association with the largest single deposition of this material in the body. Secondly, thorotrast had severely damaged the liver cells by slowly progressive necrobiosis which at the time of death was still active. The almost negligible regeneration of liver cells may also be attributed to its presence. Thorotrast had led to fibrosis of portal areas with narrowing and occlusion of veins and lymphatics and subsequent interference with the portal circulation.

In respect to the original syphilitic lesion, all that remained were coarse scarring and contractures leading to the well known hepar lobatum. That the liver was much below normal in size and weight can be explained only by the scarring and by progressive destruction of liver cells without significant regeneration.

Thorotrast in the reticulo-endothelial system of bone marrow, spleen, liver, and lymph nodes had seriously affected hematopoiesis. Much of this material was confined to the fixed cells, much of it was free, but some of it was found in circulating histiocytes, clearly indicating that thorotrast was in a continual process of migration.

Grossly, the bones were fragile and could be cut or broken easily;

histologically, there was direct evidence of necrosis where thorotrast and bone lay side by side.

In addition to these changes which were associated with thorotrast in immediate contact with tissues, there was the possible remote effect that thorotrast might have had on the cardiovascular system. Histologically, three changes were repeatedly observed in vessels in different parts of the body. First, there was degeneration and disappearance of smooth muscle fibers from the walls of both veins and arteries. Secondly, there was degeneration and interruption of the elastica in the walls of smaller arteries. Thirdly, there was a tendency on the part of arterioles and capillaries to dilate and bleed. It is of interest to correlate these changes with the clinical observation that in later years the patient's blood pressure had been consistently low.

Thorotrast as an Injurious Substance

There can be little doubt from a study of this case that thorotrast can do harm. The observations of Jacobson and Rosenbaum [5] can attest to that, for they found fibrotic changes in liver, spleen, lymph nodes, and arteries in a patient who 5 years before had received 75 cc. of this substance. Thorotrast, or thorium dioxide, is distinctly radioactive. In this respect it closely resembles uranium and radium, the activity of which depends on the continual liberation of ionizing radiations known as alpha, beta, and gamma emanations. In the body it is the gamma ray which is known to cause most harm. The amount of thorotrast injected into the body for diagnostic purposes varies, but the maximum doses employed are known to have a gamma ray equivalent to about 1 to 3 μg. of radium. The general effects of irradiation injury in the body are well known and parallel very closely the findings in our patient. For example: Bone marrow shows hypoplasia, dysplasia, and a disappearance of eosinophils; capillaries dilate and show a tendency to bleed; lymphoid tissue shrinks through injury to lymphocytes; bones become atrophic and break; glandular tissue degenerates; and smooth muscle may disappear.

Because of the property of spontaneous disintegration possessed by radioactive substances, it was of interest to know whether thorotrast, after a period of 12 years in the body, was still radioactive. To answer this question, blocks of liver tissue that had been preserved in formalin as long as 12 months after the autopsy, were tested with a Geiger-Mueller counter. This is a comparatively simple electrical apparatus, named after its inventors, for measuring and observing cosmic emanations. Each piece of liver gave a positive recording and the highest reading was obtained from the area of greatest thorotrast concentration. This was also the site of the primary malignant tumor.

Thorotrast as a Sarcogenic Agent

In 1929 Martland and Humphries [6] were the first to report the appearance of osteogenic sarcoma, attributed to radium and mesothorium, in 2 of 15 girls employed at painting watch dials with luminous radioactive paint. This report was followed 3 years later by a paper by Sabin, Doan, and Forkner [7] who described the appearance of osteogenic sarcoma in 2 of 7 rabbits, surviving 11 and 19 months respectively, that had been injected intravenously with radium chloride and mesothorium. Ross,[8] in 1936, implanted a platinum tube containing 0.1 mg. of radium under the periosteum of the rib of a rabbit, and 2 years later the tube was found encased in a large, actively growing osteogenic sarcoma. More recently, Dunlap, Aub, Evans, and Harris [9] produced osteogenic sarcoma in 9 of 13 male Wistar rats after feeding each animal 100 μg. of radium. There is no longer any question about the appearance of malignant tumors in man and animals subsequent to the injection or ingestion of radioactive substances. It is apparent, however, that in each of these cases the primary tumor has been in bone. This might suggest tissue specificity, but there is another and much more probable explanation, for Martland [10] has shown that when radium salts are ingested orally, the distribution of radium in the body is like that of lead. After absorption, it is phagocytized by cells of the reticulo-endothelial system and then it enters the skeletal system where it is concentrated and, in part at least, may be permanently retained. This is important in skeletal tumors induced by radium, for it is in bone and from bone that the osteogenic sarcomas arise.

The significance of such factors as concentration and localization of this sarcogenic agent in respect to the site and type of tumor produced has been demonstrated again by Roussy, Oberling, and Guérin,[11] who successfully produced peritoneal and subcutaneous sarcomas in 8 of 15 surviving mice that had been given intraperitoneal and subcutaneous injections of thorium dioxide. In our patient the greatest concentration of thorotrast was in the liver and it was in this organ that the primary malignant Kupffer cell sarcoma arose.

Thorotrast as a Diagnostic Aid

The most widespread use of thorotrast has been in the field of gastroenterology in the detection of diseases of the liver. In urology it seemed at first to be an ideal substance for pyelography, but soon its use was discarded because it tended to cause exacerbations of existing infections, to damage collecting tubules, to lead to obstruction, and to obscure lesions within the kidney. In certain diseases of the central nervous system it has been used in both diagnosis and localization. Here it has been injected locally, into vessels and even into the ven-

tricles; but for cerebral angiography, encephalography, and ventriculography its use has been generally condemned. It is still used, however, with great advantage, in very small quantities, to outline a cyst or abscess of the brain. Its most recent use has been in a study by Davis and Potter [12] of intra-uterine respiration and gastrointestinal activity. After injecting thorotrast into the amniotic sac it soon appears in both lungs and intestines of the fetus.

SUMMARY AND CONCLUSION

A case is reported of a patient who had been given thorotrast for the visualization of the liver. With the aid of this diagnostic procedure, combined with positive serological tests, it was possible to make an accurate diagnosis of hepatic syphilis with gumma. Following specific therapy, the patient made a clinical recovery and for 12 years lived a reasonably normal life. At the age of 70, death came suddenly. Autopsy findings confirmed the diagnosis of syphilis and in addition revealed a primary hemorrhagic endothelial-cell sarcoma of the liver, the source of fatal hemorrhage, and very widespread irradiation injury affecting particularly the liver and hematopoietic system. Evidence is produced from a study of this case to support the debatable contention that thorotrast, in sufficient quantities, as a radioactive substance, is injurious. Evidence is also produced to show that thorotrast, like other radioactive substances, in sufficient time may act as a sarcogenic agent.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

All photomicrographs were made from sections stained with eosin and methylene blue.

PLATE 95

FIG. 1. Liver, showing nests of tumor cells bathed in blood. In the lower left-hand corner there are three groups of liver cells surrounded by tumor cells and blood. The tumor cells tend to group in clusters and to cover and surround liver cells with which they come in contact. The tumor cell is often as large as a liver cell; it has little basophilic cytoplasm, that varies from a ground-glass appearance to very finely granular. Its cell membrane is indistinct and when the cells are clustered it is impossible to see the lines separating one from another. The nucleus is large and occupies most of the cell. It is lightly stained. The nuclear membrane is sharp and wire-like. In shape, the nuclei resemble potatoes. There is little chromatin and this forms coarse clumps suspended in delicate interlacing threads. Mitotic figures are very seldom seen and then they are asymmetrical and hyperchromatic. × 275.

FIG. 2. Liver. In the lower right-hand corner there are trabeculae of liver cells showing compression atrophy. The remainder of the field is made up of loose tumor cells bathed on all sides by noncoagulated blood, and of a few small clumps of degenerating liver cells—all that remains of the lobule. × 140.

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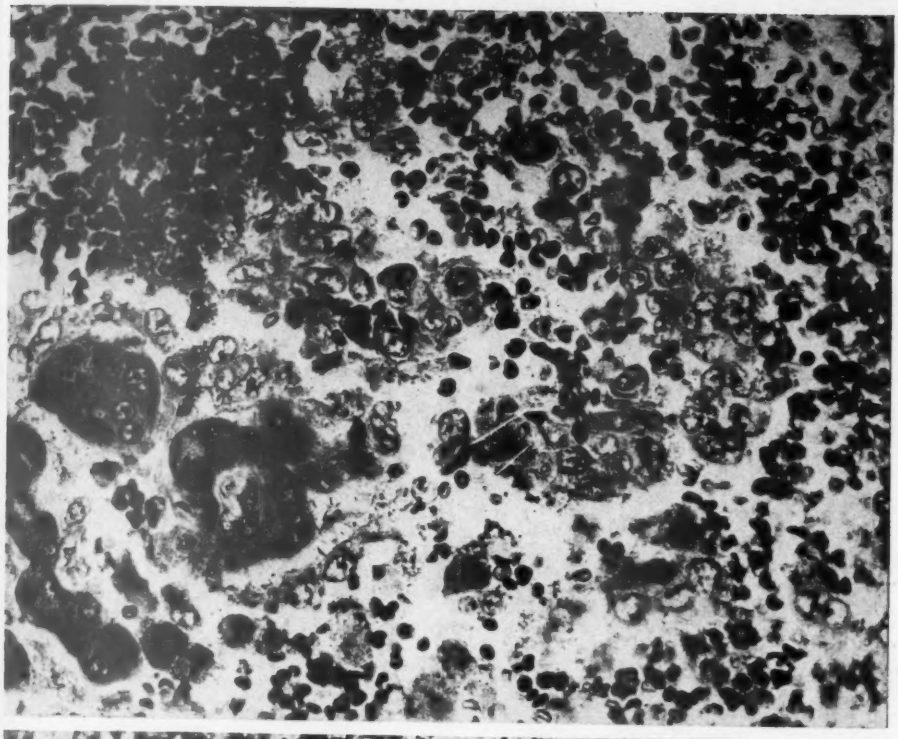

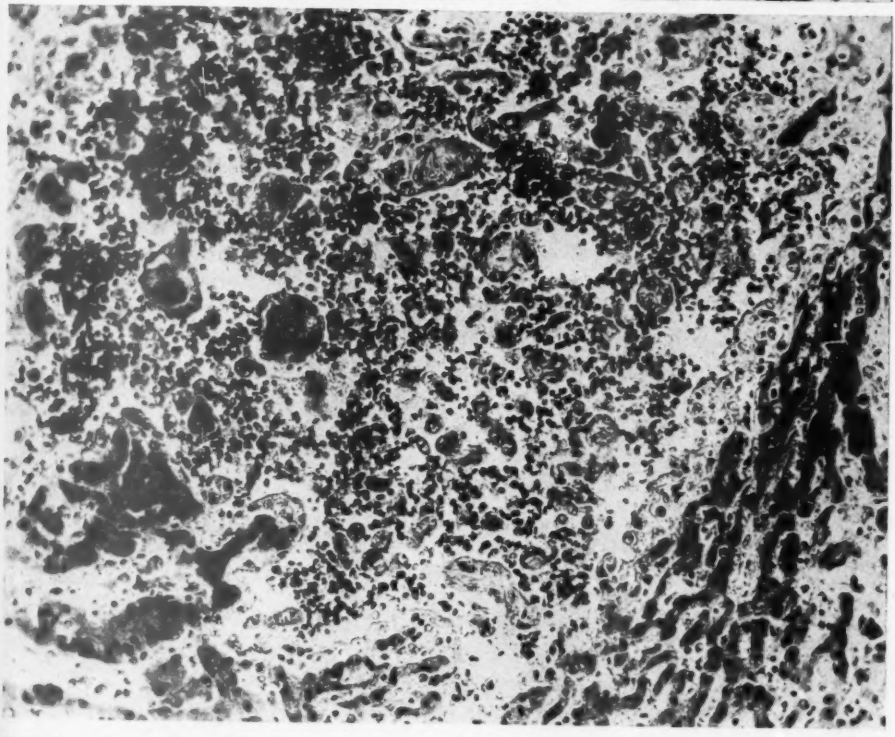

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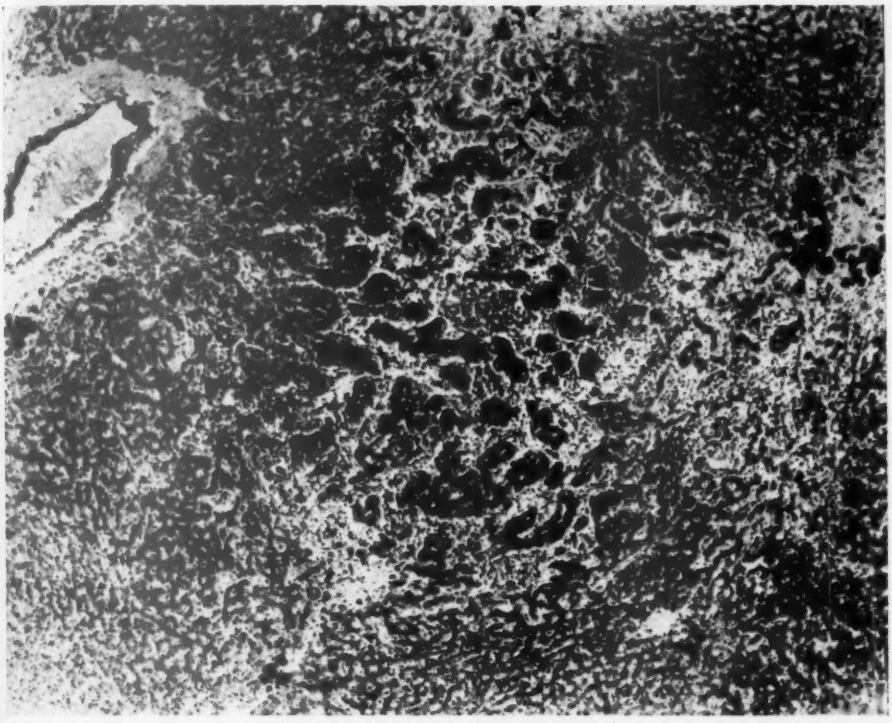
PLATE 96

FIG. 3. Liver, showing an area in which the normal trabecular pattern is broken up into small cords or islands of liver cells separated by spaces filled with blood and bordered in part by tumor cells. An occasional film of fibrin coats an island of degenerating liver cells. Small deposits of thorotrast are visible in the surrounding tissue. × 90.

FIG. 4. Liver, showing one of many hemorrhages. This hemorrhage lies very close to a portal area, below. The surrounding liver cells show compression. In the area of hemorrhage there are a number of tumor cells almost completely hidden by blood. A few necrotic and disintegrating liver cells are seen adjacent to the margin of the hemorrhage. There are small deposits of thorotrast, both to the right and left of the hemorrhage. × 140.



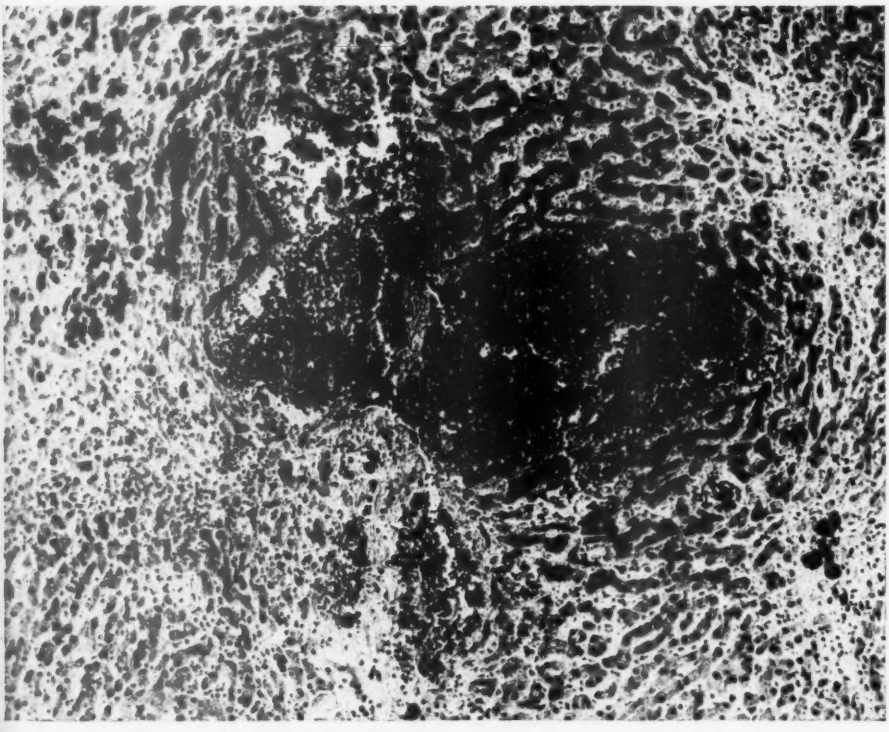


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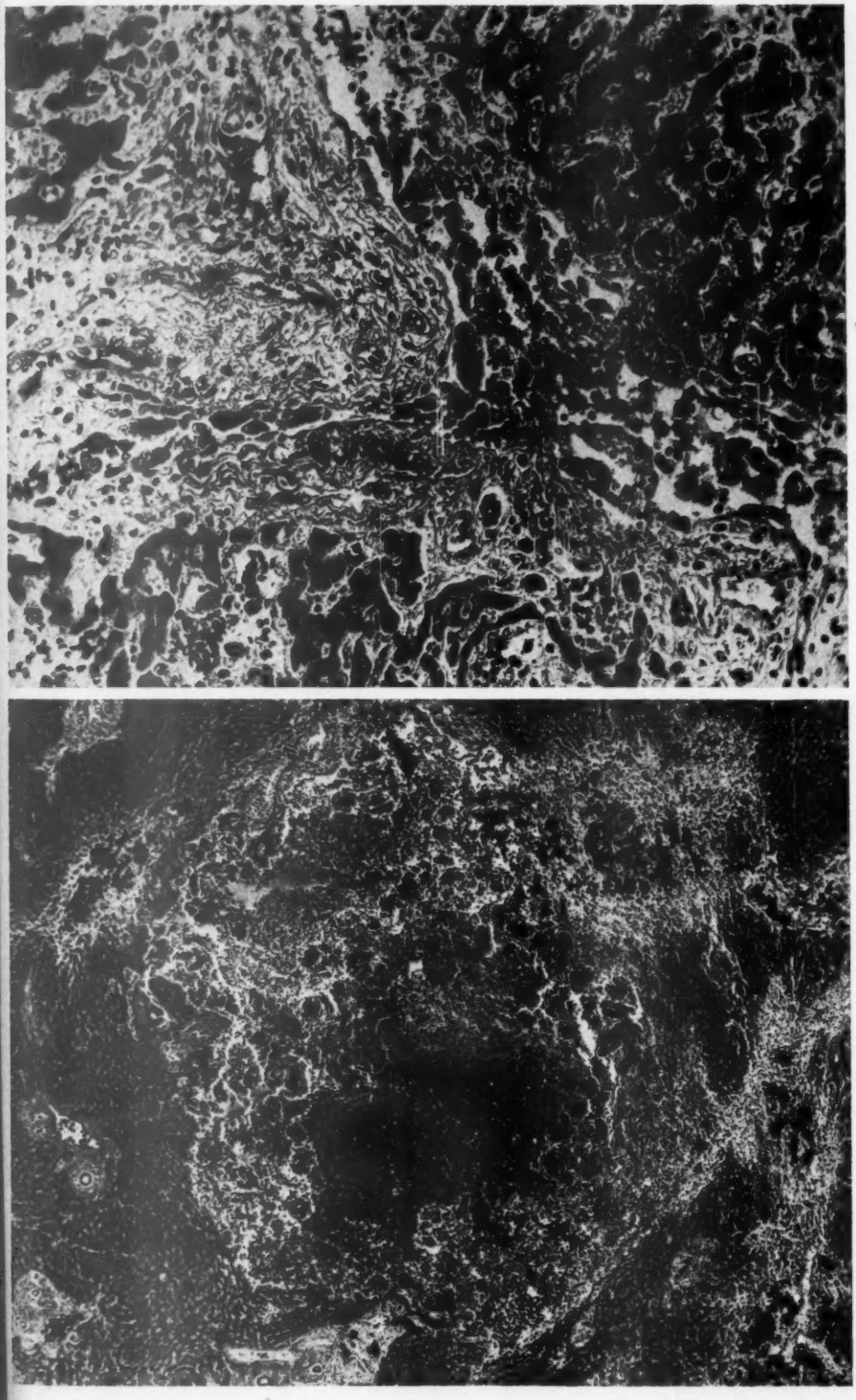
PLATE 97

FIG. 5. Liver, showing a portal area with some adjacent liver cells. A branch of the portal vein crosses and divides at about the center of the field. The lumen of the vein is dilated and almost obstructed by tumor cells. Some of these cells line the walls of the vessel, others lie free and are surrounded by blood. There is no fibrin deposition or coagulation. × 185.

FIG. 6. Liver at a very low power, showing in a single field several deposits of thorotrast, breaking up of the normal trabecular and lobular pattern of liver cells into small cords and islands, necrosis and lysis of liver cells, massive hemorrhage completely devoid of clotting, and a growth of tumor cells throughout the section. The over-all picture has a very superficial resemblance to placental tissue with its chorionic villi embedded in a sea of blood. × 45.

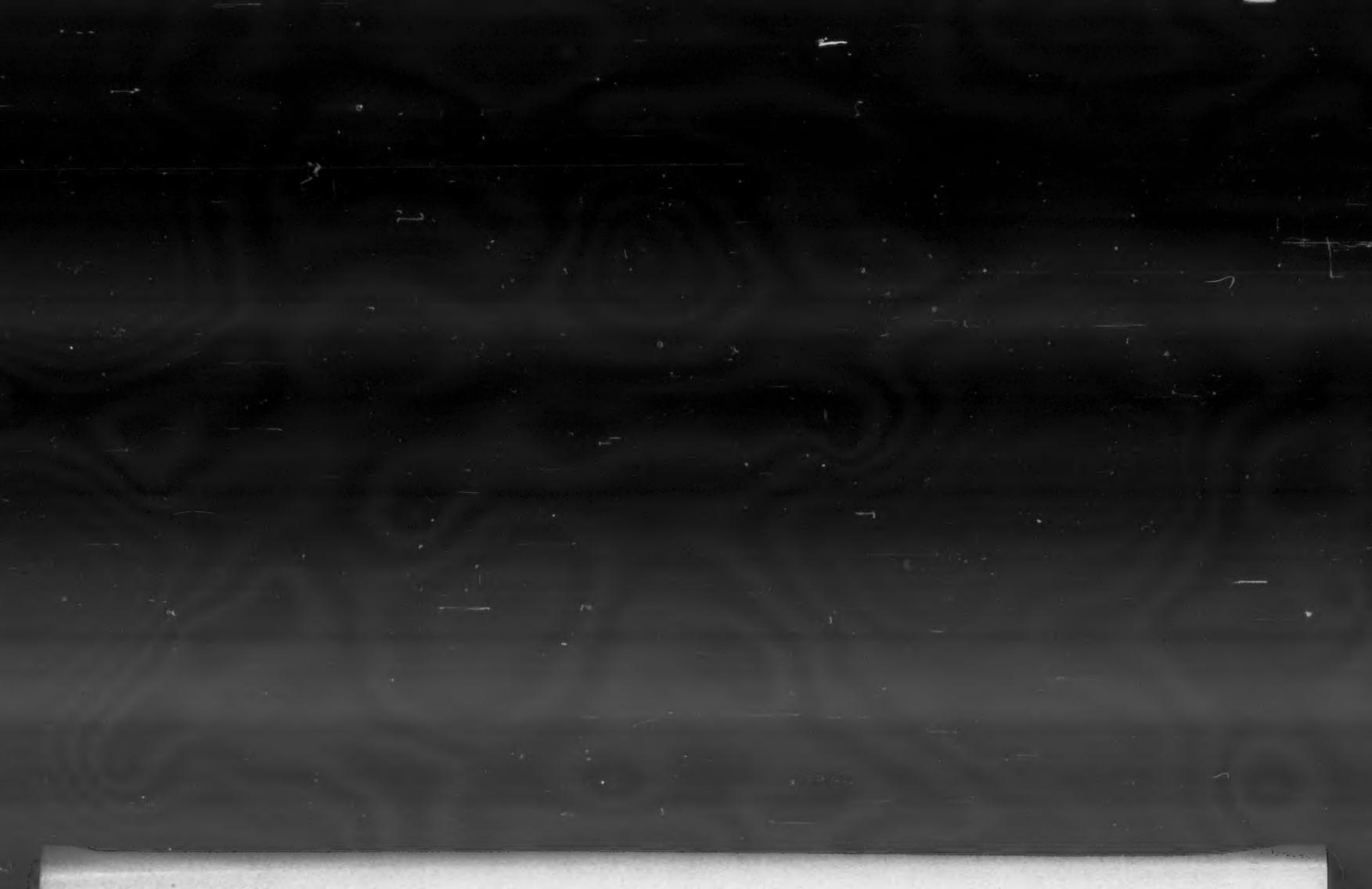


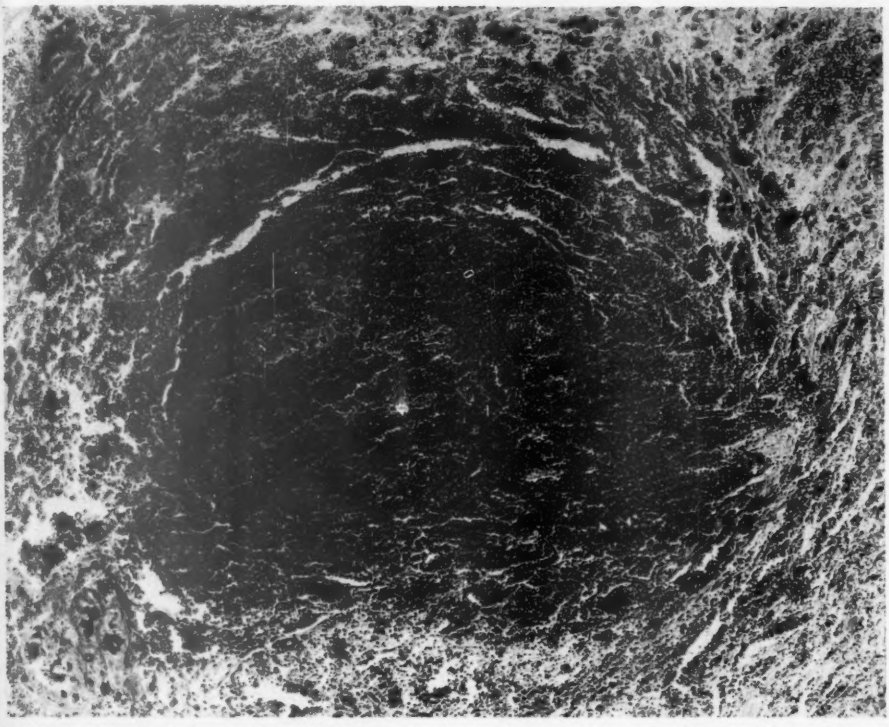
MacMahon, Murphy, and Bates Sarcoma of Liver Following Thorotrast

PLATE 98

FIG. 7. An area of the liver showing an unusually heavy deposit of thorotrast. All normal landmarks are lost and not a single hepatic cell is demonstrable. The thorotrast, like slush, lies enmeshed in poorly stained hyaline fibrous tissue. × 275.

FIG. 8. Liver, showing a central vein almost completely surrounded by a deposit of thorotrast and necrotic liver cells. This central zone is bordered by a mid-zone from which almost all liver cells have disappeared, leaving a reticulum framework infiltered with viable and necrotic histiocytes and plasma cells. Liver cells bordering this area of clearing show necrobiotic changes. There is no evidence of proliferation of liver cells in the outer peripheral zone. There is no increase in fibrous tissue or reticulum. The picture is one of destruction with little secondary resorption. × 140.





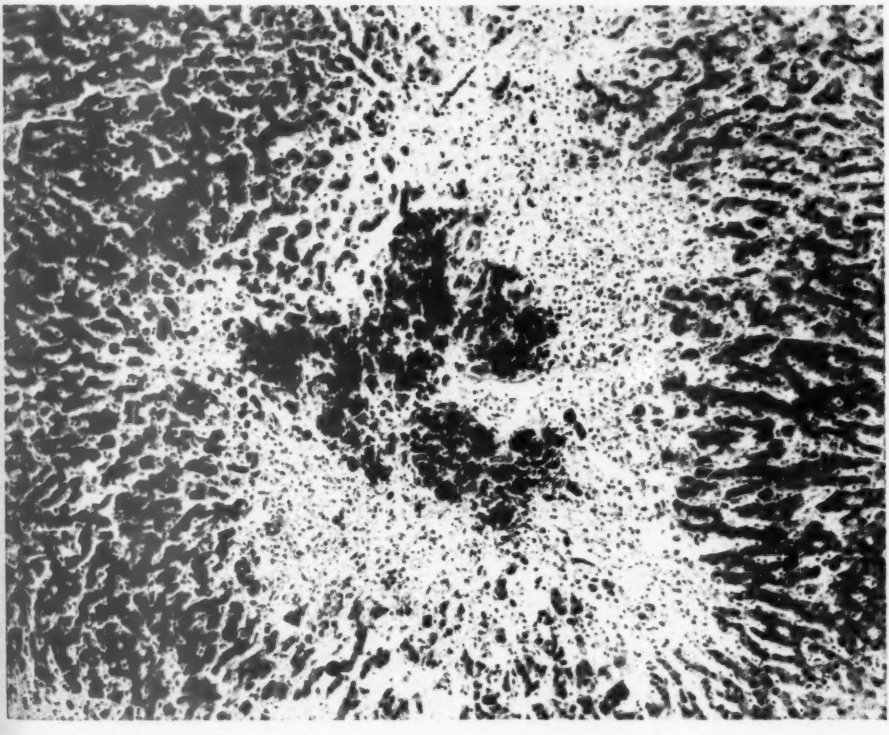


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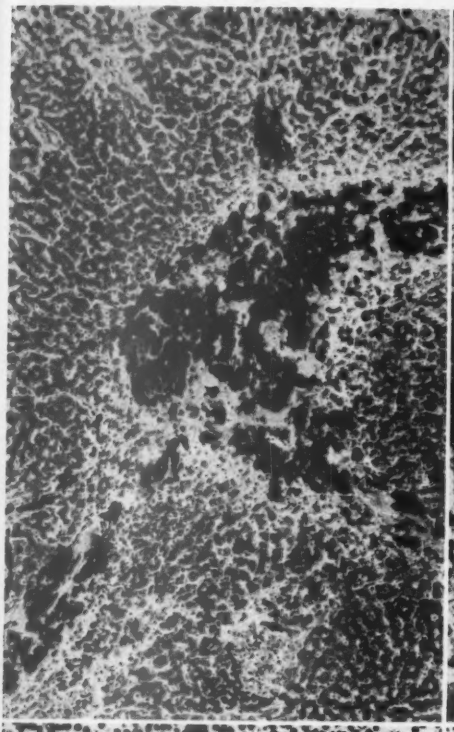
PLATE 99

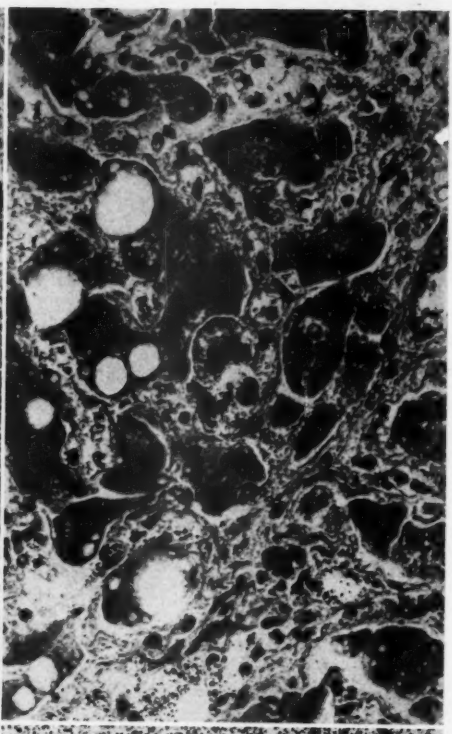
FIG. 9. Liver, showing a heavy deposit of thorotrast in the central zone of a lobule and smaller deposits in adjacent portal areas. In the large central area both liver cells and endothelial cells have disappeared. Much of the thorotrast lies loosely enmeshed in a condensation of swollen reticulum. The bordering liver cells show regressive changes and necrobiosis. Their nuclei show karyorrhexis, and fragments of chromatin are scattered throughout these cells. × 90.

FIG. 10. Liver, showing a group of liver cells undergoing fatty degeneration, hyaline degeneration, necrosis, and disintegration. There is a sprinkling of thorotrast in this field but no tumor cells are demonstrable. In the upper portion there is a light condensation of stroma as well as a scattering of lymphocytes and plasma cells where liver cells have disappeared. × 370.

FIG. 11. Section of spleen. The central portion of the field is occupied by an accumulation of thorotrast. Most of it lies free; some is confined to the cytoplasm of histiocytes, the nuclei of which it is almost impossible to identify. Red blood cells are freely scattered throughout this mass. In the periphery there are lymphocytes, plasma cells, and, in one corner, a small arteriole showing hyalinization of its wall. × 275.

FIG. 12. Section of lymph node, showing a large deposit of thorotrast. Most of this is in cells—histiocytes. An examination of the individual histiocytes containing this material shows all stages of nuclear degeneration with pyknosis and ultimate lysis. For some time the thorotrast remains within the cytoplasm of the dead cell; then, with rupture of the cell membrane, it spreads into the surrounding area. In this field there are many plasma cells, some of which are giant forms with single and multiple nuclei. Although thorotrast is never found in these cells, they show active proliferation with typical and atypical mitotic figures. In the same area there are degenerating and disintegrating plasma cells, so that the over-all number remains about constant. In this field there is little hemosiderin in histiocytes. An occasional histiocyte contains both hemosiderin and thorotrast. × 90.





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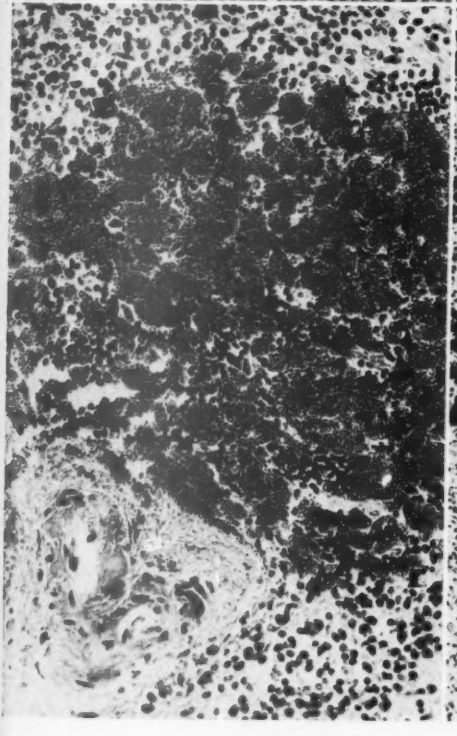

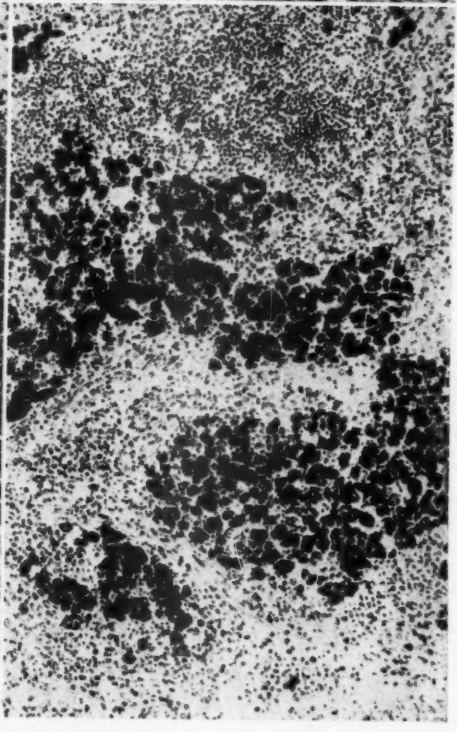

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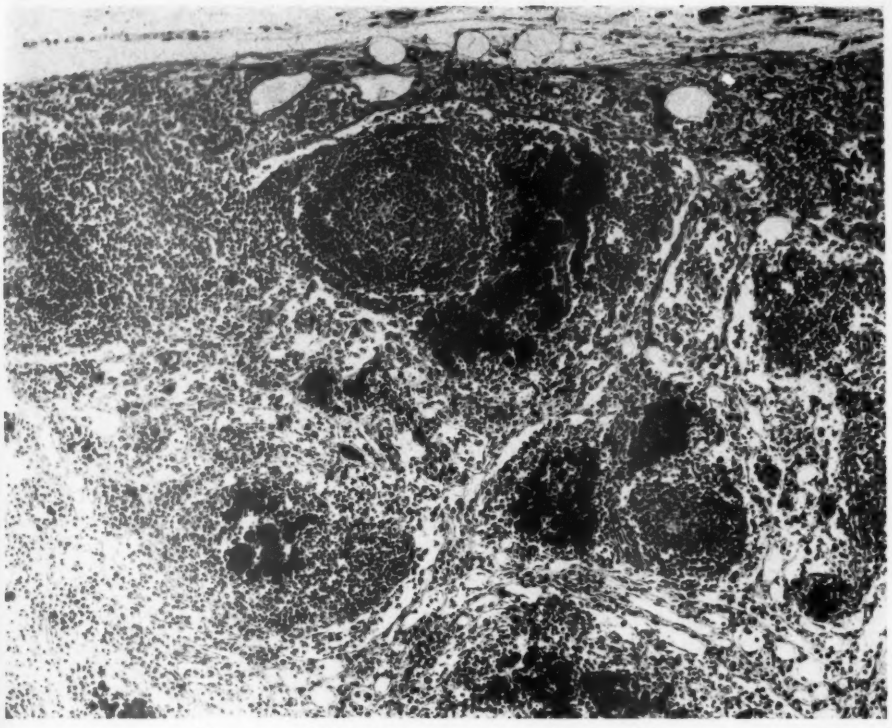
PLATE 100

FIG. 13. Section of lymph node, showing several deposits of thorotrast. Most of this is in cells but some of it is free. Histiocytes containing very small quantities of thorotrast lie free in the sinuses, indicating a continual redistribution of this material. The lymph node shows atrophy of lymphoid tissue with an absolute increase in sinus endothelial cells. Red blood cells are freely scattered throughout the section and some of these are already phagocytosed by histiocytes. Plasma cells are unusually numerous in the capsule. There is no increase in fibrous tissue. × 90.

FIG. 14. Vertebral bone marrow, showing in the center of the field a number of histiocytes singly and in clusters, laden with thorotrast. Roughly 75 per cent of the surrounding cells are erythrocytes; the rest are erythroblasts, normoblasts, megakaryocytes, myelocytes, polymorphonuclear leukocytes, and fat cells. Fluid containing threads of fibrin lies in the intercellular spaces. × 275.



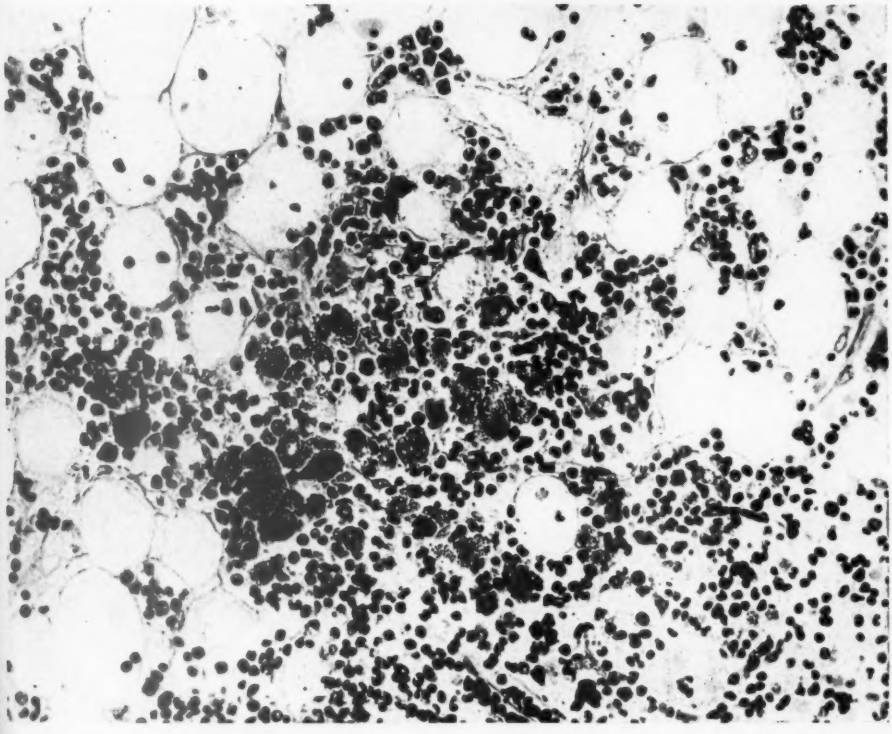


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PLATE 101

FIG. 15. Vertebral bone marrow, showing in the center of the field replacement of normal marrow by an island of mature lymphocytes loosely held together by a poorly defined, somewhat hazy, reticulum web. Red blood cells are scattered freely throughout the field. In the lower right-hand corner there is a tiny fragment of bone. × 275.

FIG. 16. Section of adrenal, showing a field from the medullary area. The pheochromocytes here are almost completely replaced by lymphocytes and small deposits of thorotrast. The wall of the central vein is infiltrated with lymphocytes. × 275.

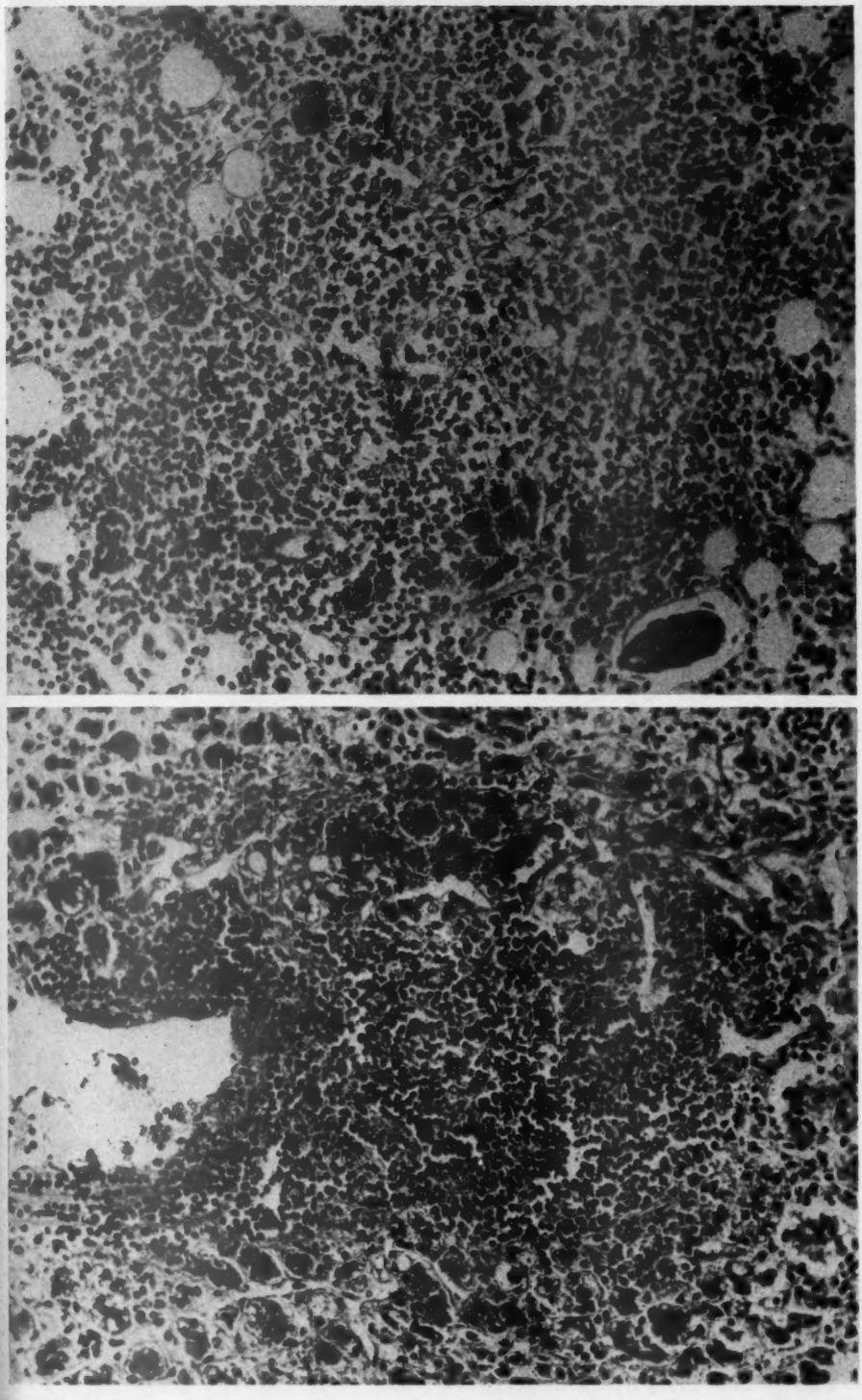

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PLATE 102

FIG. 17. Section of vertebral bone marrow. A solitary trabeculum of necrotic bone crosses the center of the field. In it there are no osteocytes; the spaces, formerly filled with bone cells, are empty. The margins of the bone are rough and its ground substance shows linear longitudinal splitting and scaling. A few histiocytes, laden with thorotrast, lie in immediate apposition to this necrotic bone. On either side are fat cells, clusters of red blood cells, and small nests of both myeloblasts and erythroblasts. × 370.

FIG. 18. Section of adrenal, showing atrophy and fibrosis of the outer portion of the glomerular zone of the cortex. In this area of fibrous tissue there are small deposits of thorotrast. × 140.

FIG. 19. Kidney. A small lobular artery crosses the center of the field. The lumen of this vessel is abnormally wide and unevenly dilated. The intima is thin and composed of a single layer of endothelial cells supported by a poorly defined basement membrane. The media is composed of atrophic muscle fibers, each of which appears as a bare nucleus bordered by a narrow rim of scarcely stainable cytoplasm. There is little edema of the surrounding stroma. The glomerulus and tubules in this field show no abnormality. × 185.

FIG. 20. Lung, showing in the center of the field a medium-sized branch of a pulmonary artery. Muscle fibers, normally conspicuous in vessels of this order, are not recognizable. The wall is composed almost exclusively of hyalinized acellular fibrous tissue. The adventitia is made up of loosely arranged collagen. The adjacent alveoli are emphysematous. × 90.

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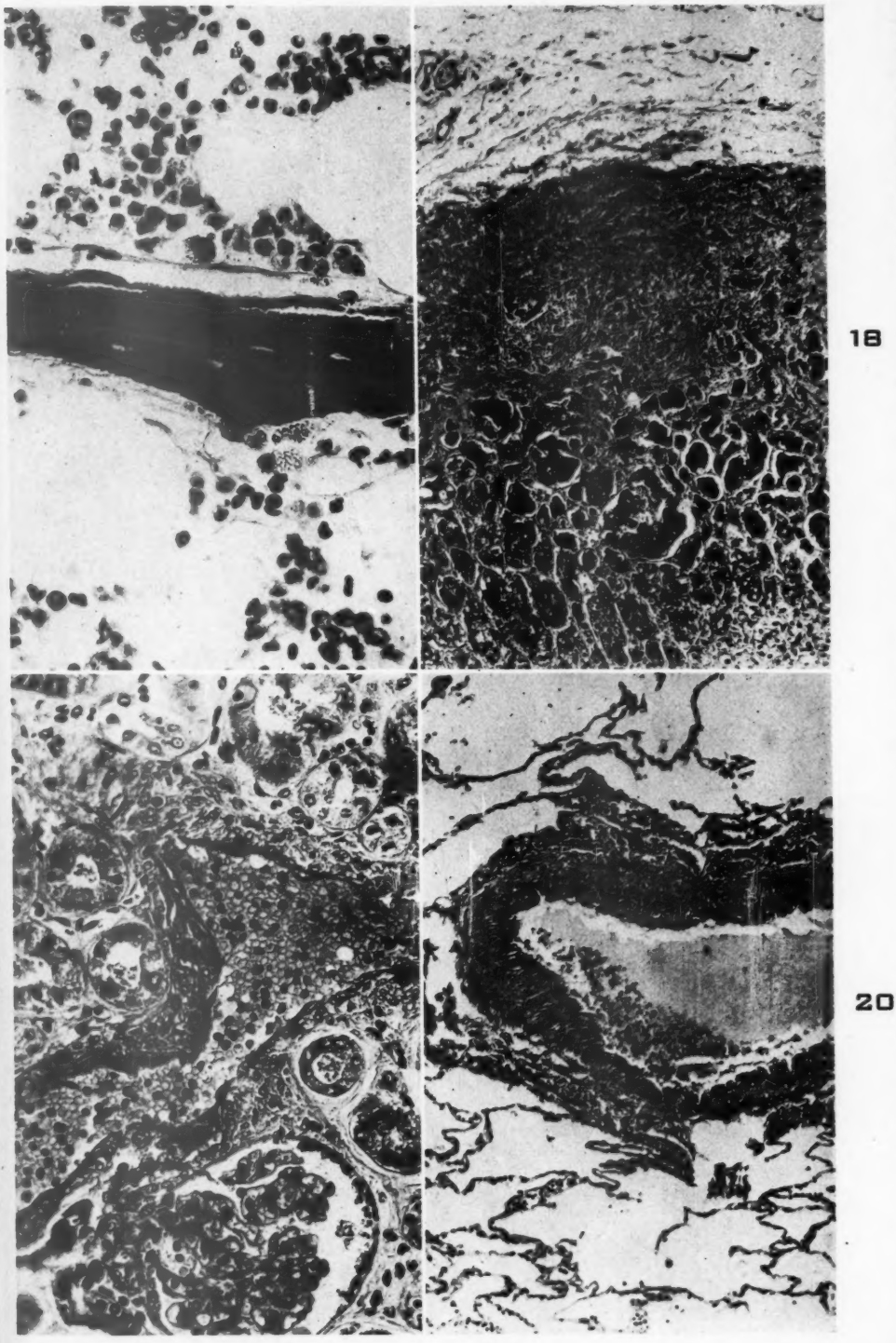

MacMahon, Murphy, and Bates Sarcoma of Liver Following Thorotrast

MYELOLIPOMA OF THE ADRENALS

Report of Seven Cases *

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After the incidental observation at autopsy of a moderately large fatty tumor in an adrenal gland, on gross examination thought to be a lipoma, which microscopically contained fat and the cellular components of bone marrow, attention was directed to the incidence and nature of this lesion.

Oberling [1] proposed the name myelolipoma, but it is questionable whether the condition is neoplastic. Gierke [2] believed that there is an immigration of elements of bone marrow at the time the epiblastic medullary tissue penetrates the mesoblastic cortex. Mieremet [3] also looked on the lesion as of embryonal origin. This would mean that it belongs in the general category of choristoma, an embryonal fault with neoplastic potentiality. Richardson [4] thought that this potentiality might occasionally be realized with formation of a true tumor. Saleeby,[5] generally in favor of ectopic or autochthonous origin, suggested the possibility that the lesion is a sort of benign bone marrow tumor, *i.e.*, a type of benign myeloma.

Arnold,[6] who reported the first case on record, looked on the lesion as an ectopia, a view shared by Kruse,[7] who found bone and bone marrow in the adrenal of a *Macacus rhesus* monkey, and shared also, apparently, by Oberling.[1]

Woolley,[8] who reported the presence of bone and bone marrow in a tuberculous adrenal, thought that metaplasia as the result of irritation was the most logical explanation. Newsam [9] found bony spicules and bone marrow, which he attributed to inflammation. Goldzieher [10] thought that the condition was due to metaplasia as the result of inflammation or cicatrization. Herzenberg [11] reported bone marrow in an accessory mass of cortical tissue attached to the hepatoduodenal ligament. She believed that there is an autochthonous development of bone marrow from capillaries, the causative insult being unknown. She compared this with the occurrence of myeloid tissue in chronic pancreatitis and in a rectus abdominis muscle. Gormsen [12] interpreted his first case in similar fashion.

Extramedullary hematopoiesis has been considered by Petri,[13] Brannan,[14] and by Gormsen.[12] Of particular interest is Gormsen's second case, a female, 53 years old, who had thrombocytopenic purpuric

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hemorrhages in many parts of the body. Minimal extramedullary hematopoiesis was found in liver and spleen, but these had been irradiated therapeutically. There was tumor-like involvement of the lower half of the right, and of the whole of the left adrenal. Microscopically, there was almost complete replacement of the cortex by immature blood cells, especially of the erythrocyte series, with numerous mitotic figures. Moderate numbers of myeloid forms were present but there were no megakaryocytes. This extended into the periadrenal fat. Gormsen interpreted this as a reaction to the profound anemia, but was not clear whether it is an accentuation of a pre-existent condition or a new focus of extramedullary hematopoiesis. Brannan, however, indicated that extramedullary hematopoiesis occurs especially in adipose connective tissue, whereas ectopic bone marrow is rare without obvious relation to anemia.

Collins,[15] in 1932, collected from the literature 15 cases of bone marrow in the adrenal and added one of his own. He gave a good resumé of the literature and analysis of these cases. He divided the cases according to the method of Soós [16] into two types. In one type the tumor is yellowish orange and on microscopic examination is found to have predominance of adipose tissue with minimal myeloid elements and a larger proportion of erythroblastic elements. Tumors of the second type are dark red or reddish brown grossly, and microscopically show predominance of the cellular elements of the bone marrow, with the myeloid elements prominent and minimal erythroblastic elements. Collins found that 4 of the reported cases were of the first type with predominance of fat, and that 8 cases, plus one of his own, showed a highly cellular picture composed of marrow elements. In one of these no fat was seen. He stated that the average age of these 15 patients was 60.1 years and that the youngest reported was 42 years, until his own, who was 32. The tumor in his case was composed of adipose tissue, with islands of myeloid tissue, the entire nodule being surrounded by cortical cells. The cellular arrangement was characteristic of bone marrow with comparative paucity of erythroblastic elements, more "lymphocytes" than are usually seen in bone marrow, numerous premyelocytes and polymorphonuclear forms, and many megakaryocytes.

Reports of Cases

Case 1

A well developed white male, 40 years old, died abruptly as the result of rupture of a saccular aneurysm situated in the anterior communicating artery of the circle of Willis. There was little arteriosclero-

sis and no other aneurysms were found. The left adrenal weighed 45 gm. and was distorted by the presence of a spherical, soft, orange yellow, tumor-like mass (Figs. 1 to 4). The opposite adrenal was normal. Microscopically, the mass was composed of adipose tissue, moderately well vascularized, and islands of bone marrow cells. All stages of granulocyte formation were present as well as a few megakaryocytes. The mass was not encapsulated and was surrounded almost completely by adrenal cortex.

Case 2

A white female, 53 years of age, died at St. Alexis Hospital, Cleveland. The main cause of death was given by Dr. John L. Work as arteriolar nephrosclerosis and terminal bronchopneumonia. The right adrenal, weighing 12.5 gm., contained a small cortical nodule. The left adrenal, which weighed 12.0 gm., contained a spherical, dark red and yellowish gray nodule measuring 6 mm. in diameter (Figs. 5 to 7). This occupied the central zone of the adrenal but was surrounded by cortical tissue. Microscopically, this was an extremely vascular nodule of red bone marrow with a small amount of fat in the stroma. It was cellular, nonencapsulated, surrounded by cortex, and contained well defined erythropoietic foci and scattered myeloid elements.

Case 3

A white male, 59 years old, died at the University Hospitals of Cleveland (autopsy no. 7735), of myocardial infarction with stenosing coronary sclerosis. The right adrenal weighed 11.2, the left, 17 gm. In the left gland was a well demarcated, reddish brown nodule which measured 7 by 7 mm. The proportion of cortical tissue to medulla was markedly increased. Microscopically, the nodule in the left adrenal showed a richly cellular structure with a moderate amount of fat in the stroma. The picture was typical of red marrow, with megakaryocytes and all gradations of red and white cell formation in abundance (Fig. 11). The mass was without capsule, occupied the central part of the gland, and was surrounded by cortical tissue. Yellowish brown pigment granules were scattered in the stroma and in phagocytes.

Case 4

A colored female, 51 years of age, died of a cardiorenal syndrome, the heart weighing 825 gm. (autopsy no. 13246, Cleveland City Hospital). The left adrenal weighed 10, the right, 15 gm. In the latter there was an oval, fatty mass, 3 by 2 cm., surrounded by thinned cortex (Fig. 8). The center of the mass was composed of dark reddish brown, somewhat friable material, around which the periphery of the nodule

was yellowish gray. Microscopically, this mass was an extremely vascular, partially encapsulated, red marrow focus with fibro-adipose stroma rich in thin-walled blood vessels and sinusoids (Figs. 9 and 10). In the dense parts of the stroma were occasional spicules of bone. Cortical tissue almost completely surrounded the tissue mass and in some places the margin of the focus faded insensibly into an excessively vascular cortex. The marrow elements were pleomorphic, including all myeloid elements, megakaryocytes, and erythropoietic foci. Sicklemia was present.

Case 5

A white male, 76 years old, died of confluent bronchopneumonia (autopsy no. 11939, Cleveland City Hospital). He had arteriosclerosis and his heart weighed 500 gm. The right adrenal was normal. In the left adrenal was a moderately firm, spherical mass, 1 cm. in diameter, with a moist, yellowish gray cut surface, largely surrounded by cortex. Together the adrenals weighed 16 gm. Microscopically, the nodule had a fibro-adipose stroma with the connective tissue more prominent than in the usual lipoma. There was a large, dense, partially hyalinized zone of connective tissue in the center of the mass. The tissue was well vascularized, nonencapsulated, and in the stroma between fat cells there were a few young granulocytes and some erythroblastic cells in various stages of development. There were more erythropoietic than myeloid elements.

Case 6

A white male, 62 years of age, died of confluent bronchopneumonia, but with cardiorenal disease. The heart weighed 690 gm. (autopsy no. 6832, University Hospitals of Cleveland). The right adrenal weighed 9, and the left, 7.5 gm.; and in the left was a spherical, firm, homogeneous, grayish white nodule measuring 8 mm. in diameter. This was not encapsulated and occupied the central zone, being surrounded by cortex. There was no medulla at this site. Microscopically (Fig. 13), there were various erythropoietic elements and many blood sinusoids between the fat cells. Myeloid tissue elements were scanty. Many phagocytes were present; some were vacuolated and some contained yellowish brown pigment granules.

Case 7

A white female, 64 years old, died because of a massive ventral hernia with gangrene, perforations, and peritonitis (autopsy no. 14246, Cleveland City Hospital). Together, the adrenals weighed 15 gm. In the left was a large nodular tumor which was almost homogeneous, grayish yellow, and surrounded by cortex. It was ovoid and measured approximately 2 by 1.5 by 1 cm. Microscopically, it was

composed largely of fat with more vascularization than the usual lipoma (Fig. 12). There were numerous thin-walled blood sinusoids, some of which were large. In these were scattered foci of small cells with dense, often lobulated nuclei and pink, scanty cytoplasm. These showed the structure and cellular constituents of erythropoietic centers. Few myeloid elements were present. The tumor was not encapsulated.

Discussion

These 7 cases were interpreted as showing foci of true bone marrow arranged in nodular or tumor-like form. In the course of study of this material many other adrenals were found which contained small amounts of adipose tissue. In some there were foci of cells foreign to the usual fat tissue, such as small and large nongranular mononuclear cells. In addition there were found four fatty tumor masses, with few or no myeloid elements, which were interpreted as lipomas. An additional similar case has been found recently in material at the Youngstown Hospitals. However, none of these fatty tumors was encapsulated and the vascularity and amount of stroma between the fat cells were more than is common in lipomas found elsewhere. The general structure was similar to that interpreted as fatty bone marrow except for paucity or absence of myeloid elements. Two hard tumor masses were encountered which showed a large deposit of brown pigment, calcium, and bone formation, but few or no myelocytic cells. These seem to have followed hemorrhage or infarction. In one of these the patient had multiple infarcts in other organs.

In all 7 cases there was some degree of fat in the stroma. Moreover, most or all of them were nearly or completely surrounded by adrenal cortical tissue. Cases 1, 5, 6, and 7 seem to fit into type I of Soós [16] with predominance of adipose tissue, minimal myeloid elements, and prominent erythropoietic tissues. Cases 2, 3, and 4 fit Soós' type II, showing a richly cellular, red marrow structure with prominent myeloid elements and relatively fewer erythroblastic elements. The average age of the patients was 57.9 years, the youngest being 40 years old. Four were males. All were observed incidentally at autopsy and the patients had no symptoms or signs attributable to the adrenal lesions. The division into the two types of Soós is somewhat artificial because these 7 cases show gradations of one type into the other. Even those lesions which might be called lipomas have more stroma, richer vascularization, and greater numbers of cells than is common in lipomas elsewhere.

Woolley [8] listed three hypotheses to account for heterotopic bone formation, as follows:

1. Embryonal rests of osteogenic tissue, misplaced during develop-

ment, which, with proper stimuli or changed physiologic conditions, produce bone or marrow.

2. Embolism of bone marrow cells by way of the blood stream.

3. Metaplasia, which holds that any cell under certain conditions may change its morphologic features and become physically and perhaps chemically like other cells originating from the same embryonal layer.

Woolley [8] called attention to the relationship between blood supply and bone formation, which many have investigated. He stated that ligation of renal vasa frequently results in bone or marrow formation, although in other organs only necrosis and calcification result. In the ordinary lipoma seen in surgical material the blood supply seems scanty and yet ossification is rare. In the adrenals containing these fatty nodules the blood supply seems abundant although the rate of flow may be slow. In my material the more cellular nodules were richly vascular, and in these the granulocytes were prominent. This perhaps fits in with the statement of Sabin [17] that widely dilated sinuses give abundant and slow blood flow, which brings the maturation factors in the right concentration for granulocytes between these vessels. She stated that for red cell formation collapsed capillaries with low oxygen tension are the most favorable and that the red elements are formed within the blood vessels.

The view that the lesions represent extramedullary hematopoiesis is not borne out by these cases, for in none of them was anemia a conspicuous feature.

The blood vessels are of mesoblastic origin and Kruse [7] suggested that in the course of development certain of the primitive cells become osteoblasts and hematoblasts instead of vascular endothelium.

Summary

Seven examples of foci of bone marrow in the adrenal are reported, of which four represented yellow, and three red marrow. The features are similar to those reported in the literature. Although no positive conclusions as to origin can be drawn, the theory of Gormsen that they are derived from proliferation of the reticulo-endothelial cells of the blood sinuses is attractive. However, the stimulus which causes these cells to differentiate toward fat and bone marrow, in such a degree as to produce grossly visible nodules, has not been disclosed.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 103

FIG. 1. Case 1. Rounded, orange-yellow, fatty mass in the left adrenal weighing 45 gm. The tumor is surrounded by a thin rim of flattened cortex.

FIG. 2. Case 1. Margin of the tumor to show rim of cortex, absence of capsule and cellular marrow tissue in fat stroma. × 163.

FIG. 3. Case 1. Clumps of cells of hematopoietic series in vascular area with some fat. × 640.

FIG. 4. Case 1. Area showing two megakaryocytes and developmental forms of granulocytes as well as a hematopoietic focus at the lower left. × 350.

FIG. 5. Case 2. In this adrenal (left) the nodule is dark red, homogeneous, surrounded by cortex but without a capsule. It is in an adrenal which is otherwise normal.

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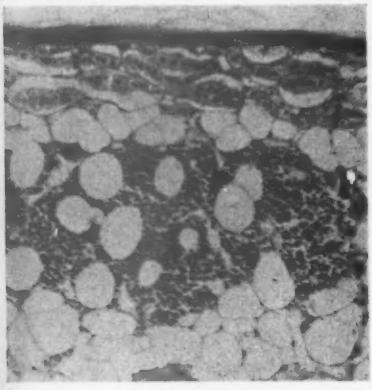

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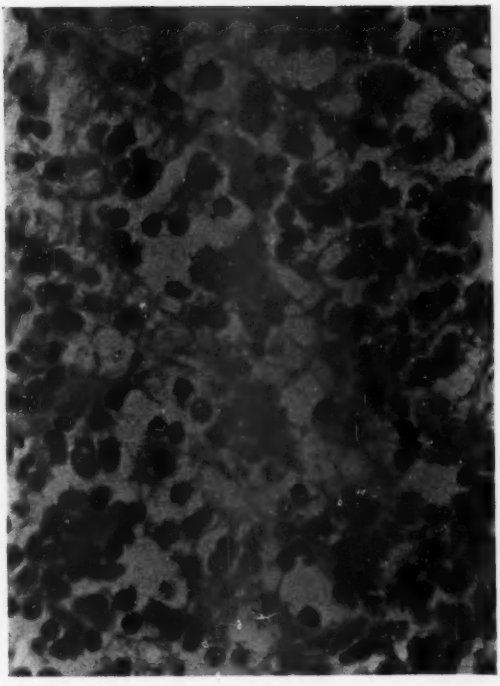

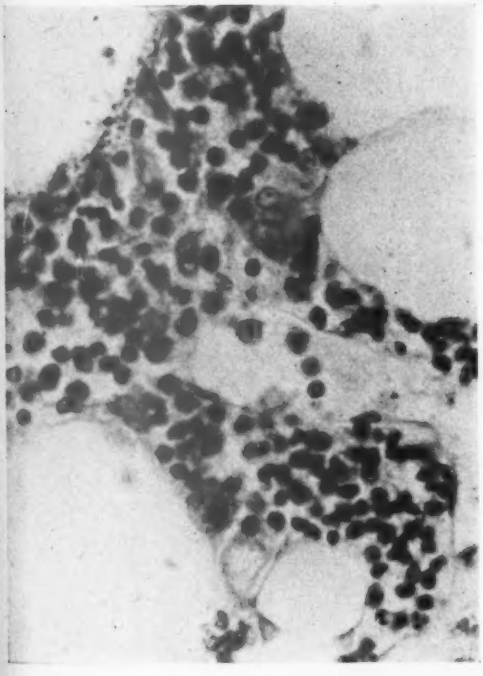

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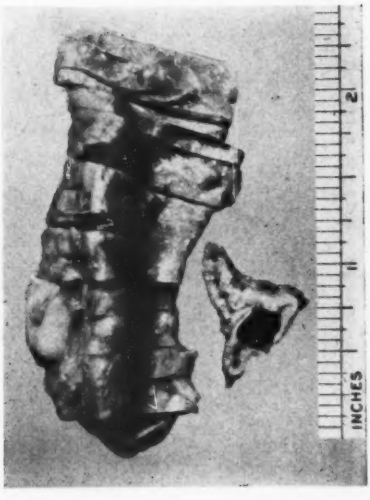

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Myelolipoma of the Adrenals

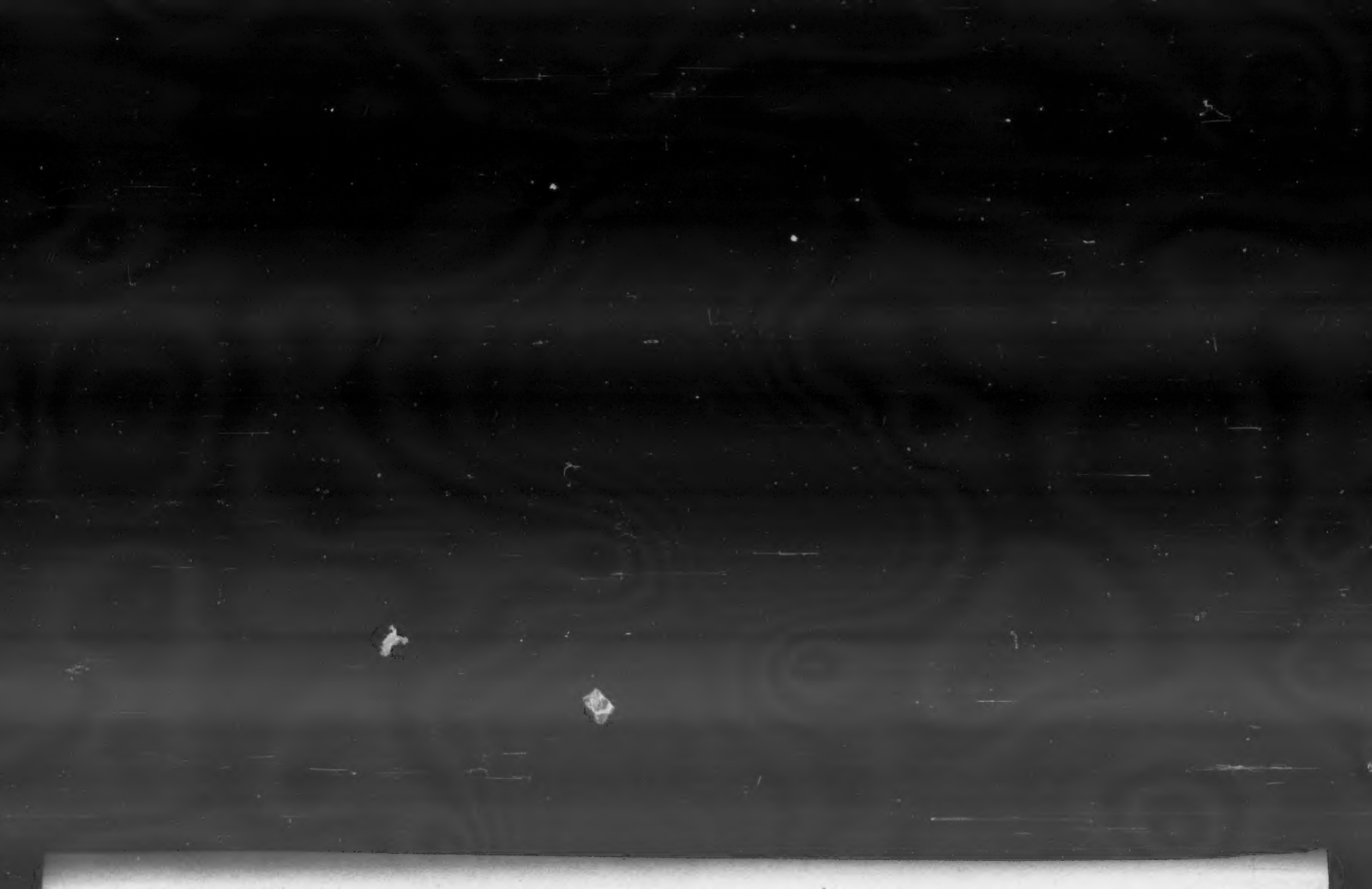
PLATE 104

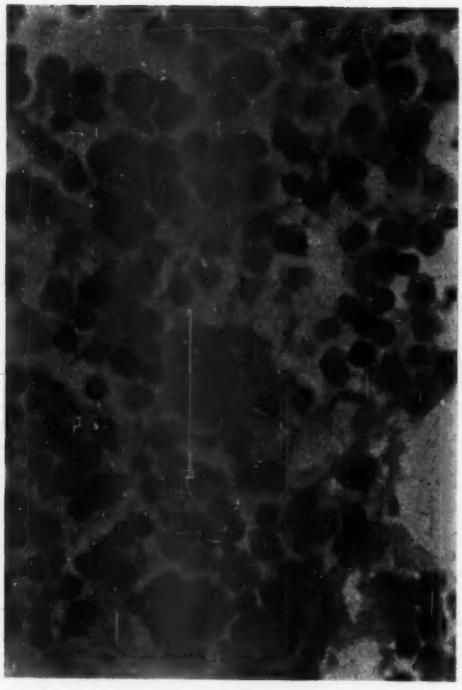
FIG. 6. Case 2. An area in the adrenal tumor in which hematopoiesis predominates but developmental forms of red and white cells are represented. × 540.

FIG. 7. Case 2. Higher power view of the same lesion as in Figure 6, to show megakaryocytes and all other elements of red bone marrow. × 640.

FIG. 8. Case 4. Photograph of the right adrenal showing poorly defined masses scattered in the cut surface. The tumors were similar microscopically, with a fatty stroma, focal fibrosis, calcification, and ossification. The masses are extremely vascular with cellular constituents pleomorphic.

FIG. 9. Case 4. Low power magnification to show the moderately fibrous zone with little fat and abundant hematopoietic activity. × 213.





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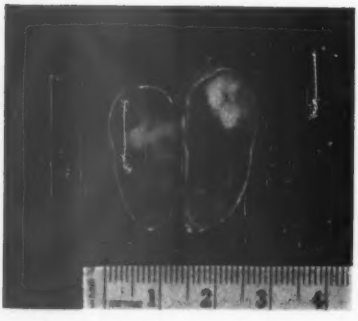

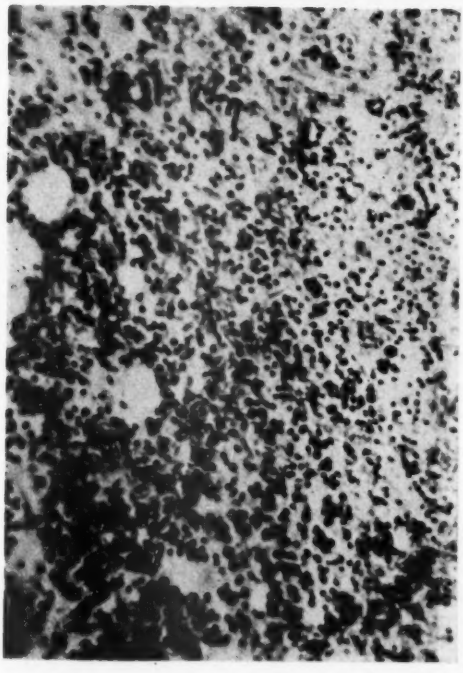

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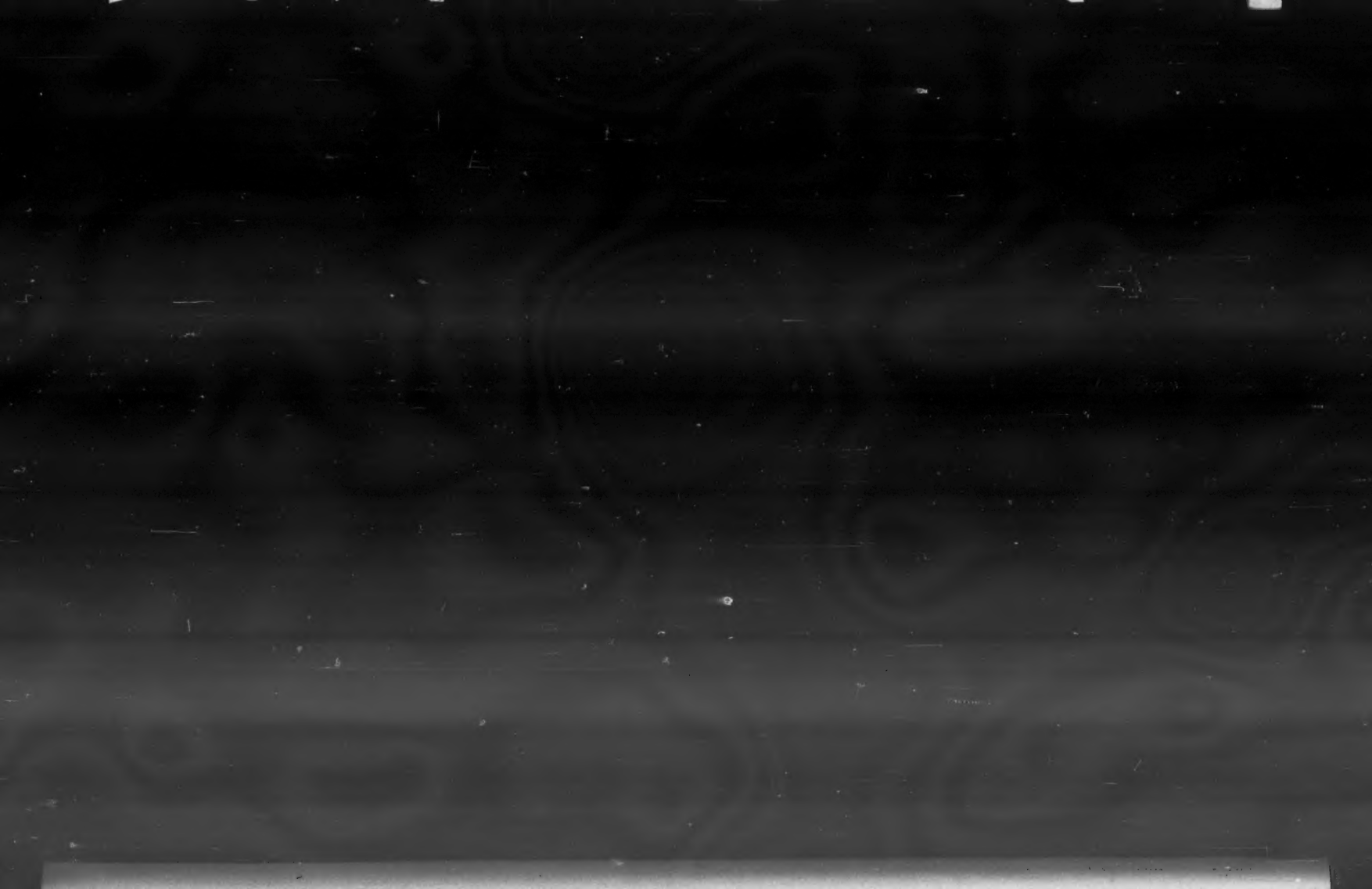
PLATE 105

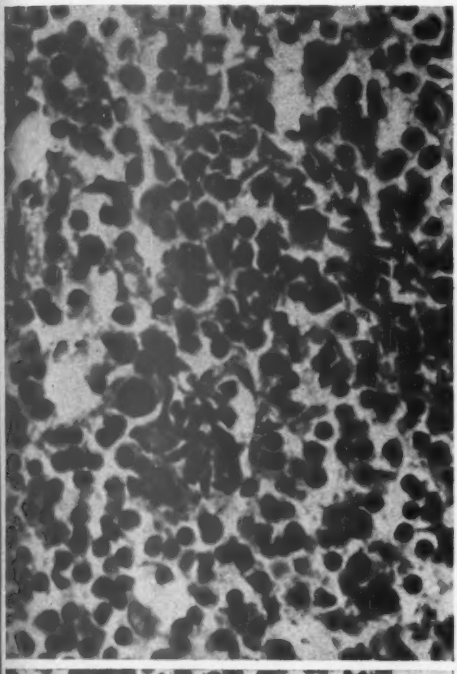
FIG. 10. Case 4. Pleomorphic zone of the adrenal tumor, in which there are atypical megakaryocytes and scattered granulocytes. The dark cell in lower left center is multinucleated with overlapping nuclei. × 615.

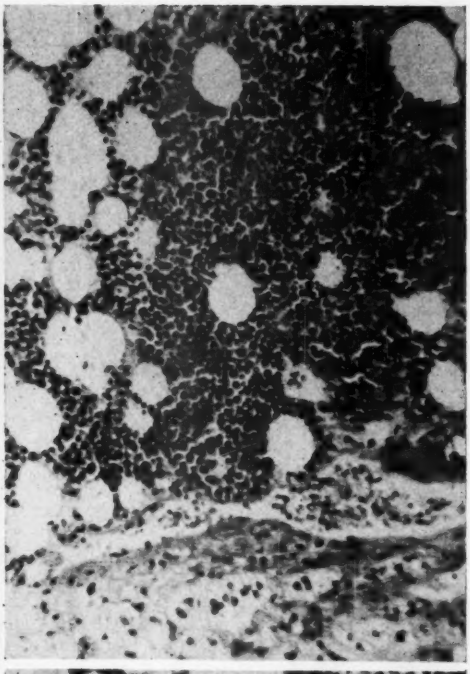
FIG. 11. Case 3. Photomicrograph to show the outer part of the red marrow mass with fat in the stroma. Both granulocytic and erythrocytic cells are abundant, but no megakaryocytes are seen. × 164.

FIG. 12. Case 7. Photomicrograph of the grayish yellow mass in the cortex. Stroma is fatty with moderate vascularization, and foci of erythropoiesis are scattered in the vascular channels. × 141.

FIG. 13. Case 6. Photomicrograph of tissue from the firm grayish white nodule in the left adrenal, showing a cellular focus in an extremely vascular fatty stroma. Here are seen mostly erythropoietic cells in dilated blood sinuses. There are also scattered cells of the granulocytic series in the stroma but no megakaryocytes are found. × 665.







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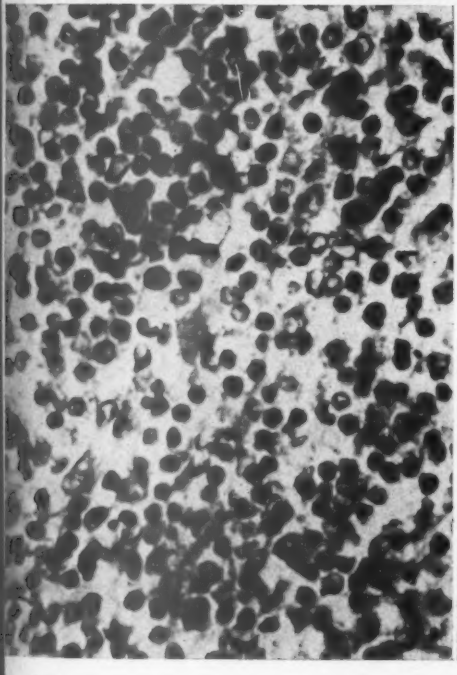

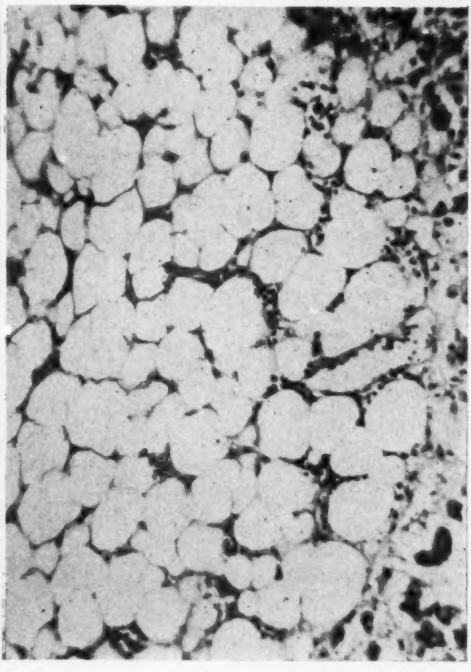

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Myelolipoma of the Adrenals

OBSERVATIONS ON TYZZER'S DISEASE IN MICE *

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A spontaneous disease of Japanese waltzing mice characterized by large focal inflammatory lesions of the liver was noted and described by Tyzzer [1] in 1917. His excellent photomicrographs illustrate the long, banded, bacilliform organisms found in affected cells of the liver and intestinal mucosa. Although the organism associated with the disease could not be cultivated, it was regarded as the etiological agent and called *Bacillus piliformis,* solely on morphological grounds. Experimental transmission of the disease to normal waltzing mice was accomplished with some regularity by contact with diseased animals or by the ingestion or intravenous injection of very large doses of infected tissues. However, the appearance of the disease in various control groups indicated its general presence throughout these transmission experiments. Ordinary laboratory animals were not susceptible, but the disease was later found by Tyzzer [2] in certain inbred strains of mice being used for cancer studies.

A disease apparently identical with that described by Tyzzer was encountered in a British strain of Swiss mice during the functional activities of the First Medical General Laboratory, U. S. Army, in England. The present report reviews briefly the natural infection and the characteristics of the associated organism; in addition, it describes for the first time the regular and consistent experimental transmission of this disease in mice and other laboratory animals, and the cultivation of the organism in tissue cultures.

MATERIALS AND METHODS

Laboratory Animals. The laboratory animals employed in these studies, including the Swiss mice in which the disease was enzootic, were obtained from the British Agricultural Experiment Station at Compton, England. The farm strain of mice was an inbred stock which had originated from the cross breeding of albino with wild mice.

Culture Media. The basal medium employed in the present work was standard beef heart infusion broth prepared from the dehydrated powder † or from fresh material.[3] This broth base contained 0.8 per

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† Difco Laboratories, Detroit, Michigan.

cent NaCl and 1 per cent of neopeptone or proteose peptone. Various combinations of blood, serum, glucose, ascorbic acid, and thioglycollate were added to this medium for enrichment. The Löffler's, Löwenstein-Jensen's, and Sabouraud's media were the standard preparations used in diagnostic bacteriology.[3]

Cultural Methods. Cultures were incubated at 37.5°C. except in a few instances when parallel cultures were carried at room temperature (20 to 23°C.). An atmosphere of 10 to 12 per cent CO_2 was obtained by the candle jar method. A McIntosh-Fildes' jar was used to obtain anaerobiosis which was checked by a methylene blue-alkaline glucose control tube.

Tissue Cultures. Agar slope tissue cultures were prepared according to the method described by Zinsser, Wei, and Fitzpatrick.[4] The minced tissues of either 10-day-old chick embryos or of 15 to 20-day-old mouse embryos served as a source of cells. A 10 per cent suspension of infected mouse brain was added to the embryonic tissue and the mixture was spread on serum agar slopes; the tubes were stoppered with rubber plugs and incubated aerobically at 37°C. for 5 to 10 days.

Histological Methods. Tissue specimens for microscopical examination were fixed in Zenker's formalin solution; paraffin sections were stained with hematoxylin and eosin, by Giemsa's method, or by Weigert's modification of Gram's stain.

Experimental Findings

The Natural Infection

Outbreaks of the natural infection occurred in the mouse colony in the late winter and early spring of 2 successive years. The epizootic was associated in each instance with the overcrowding of breeding stocks. Mice which succumbed to the disease showed only vague, indefinite signs of illness consisting of a ruffled coat, an emaciated appearance, and occasionally a mild diarrhea. The autopsy findings, which were essentially the same as those described by Tyzzer, revealed a liver mottled by large (1 to 2 mm.), hard, yellow, inflammatory lesions with punctate necrotic centers. These were visible through the abdominal wall after the skin had been reflected. No other macroscopic lesions were observed consistently. Histological examinations of the hepatic lesions showed that the characteristic structure consisted of a central area of necrosis frequently surrounded by a zone of infiltrating polymorphonuclear cells with a peripheral area in which the parenchymatous cells had undergone some necrobiotic changes (Fig. 1). In this marginal zone, hepatic cells were seen frequently with their cytoplasm

filled with the organisms associated with the disease. These organisms were regularly demonstrated microscopically in stained impression smears of infected tissue and in smears of suspensions of ground tissue. They appeared as gram-negative, long, thin, nonbranching, multiple, banded rods which were often fusiform (Fig. 4). Although pleomorphic bulbous structures of the type described by Tyzzer were seen occasionally, we were not convinced that they warranted a classification of the organism as a member of the spore-forming group. Darkfield examinations of infected tissue suspensions failed to demonstrate motility.

Experimental Transmission of the Disease in Mice

Attempts were made to transmit the spontaneous disease of Swiss mice to apparently healthy mice of the same stock with suspensions of affected liver tissue. In one series of experiments, groups of 10 mice were either forcibly fed or inoculated by the intraperitoneal, intravenous, or subcutaneous route with such suspensions. No significant increase in incidence of hepatic disease was found in the treated groups during a period of 40 days' observation, when compared with the untreated control group. In contrast, the intracerebral inoculation of mice with suspensions of affected liver tissue resulted in each of three instances in the production of an acute encephalitis in the treated animals. The experimental disease was transmissible by intracerebral injection of 10 per cent suspensions of brain tissue from infected mice. One strain, called ML-7, was maintained in this manner through 100 serial transfers; the work with this strain provided most of the material for the present study. This method of producing an experimental encephalitis is important for the further investigation of the disease since transmission by other methods has not been satisfactorily consistent, and the etiological agent does not grow on ordinary culture media.

The Experimental Encephalitis in Mice

Swiss mice inoculated intracerebrally with suspensions of infected tissue usually died with signs of disease of the central nervous system between the third and the eighth day after injection. Affected animals developed increased irritability and paralysis of one or more limbs followed by convulsions and death, all in the course of a few hours. Neither the brain nor the viscera of mice dying with encephalitis showed macroscopic lesions. Histological examination of affected brains revealed areas of complete liquefaction necrosis which were sometimes surrounded by a moderate infiltration of polymorphonuclear cells (Fig. 2). The characteristic banded organisms were frequently

seen at the periphery of the necrotic foci; these were generally in an intracellular position (Fig. 5).

The Susceptibility of Other Laboratory Animals

Farm mice, white rats, rabbits, and hamsters were tested for their susceptibility to the experimental encephalitis. Farm mice were as susceptible to this infection by the intracerebral route as were the Swiss mice from which the agent originally had been recovered. Rats, rabbits, and hamsters also developed encephalitis when injected intracerebrally with 10 per cent suspensions of brain tissue infected with the agent of mouse liver disease. Microscopical examination of the brains of these animals revealed the presence of lesions similar in all respects to those found in experimentally infected mice. Figure 3 illustrates a lesion in the brain of an infected hamster.

Cultivation of the Piliformis Organism

The cultivation of the piliformis organism on cell-free media has been unsuccessful in our hands. In addition to the ordinary bacteriological culture media, a number of enriched media were employed under various environmental conditions. Certain of these media were of the type ordinarily employed for the cultivation of pleuropneumonia organisms. Preparations of such inoculated fluid and semisolid media were stained by Giemsa's as well as Gram's method for microscopical examination. A summary of the work is presented in Table I.

Some success was achieved, however, when the cultivation of this agent was attempted in serum agar slope, tissue cultures. Tissue cultures were examined microscopically for evidence of growth of the typical organisms and were tested for the presence of bacterial contaminants by the routine inoculation of blood agar plates. In addition, the pathogenicity of these tissue cultures was determined by the intracerebral inoculation of mice. Only slightly encouraging results were obtained when the cultivation of the piliformis organism was attempted on tissues from minced chick embryos. In three separate series of experiments, a moderate number of morphologically typical organisms were present in the primary culture and these proved to be virulent for mice; subcultures, however, contained only a few organisms and were completely avirulent.

In contrast, the use of embryonic mouse tissue in the agar slope cultures met with more success. Cultivation in this medium resulted in an abundant growth of the piliformis organisms which appeared typical of those seen in stained smears of material from the natural or experi-

mental lesions in mice. Materials from the original and first subculture were virulent for mice, and microscopical examination of the brains of the animals which succumbed showed that they contained piliformis organisms. Although the second, third, and fourth subcultures were rich in organisms, they failed to induce obvious disease in mice. Identical results were obtained on another occasion in a similar series of cultures.

TABLE I

Culture Media Used in Attempted Cultivation of the Piliformis Organism

Base	Medium	Enrichments	Ph	Atmospheric environment	Period observed (*days*)
Beef heart infusion	2% agar	8% sheep blood	7.2–7.6	Air	10
Beef heart infusion	2% agar	8% sheep blood	7.2–7.6	10% CO_2	10
Beef heart infusion	2% agar	8% sheep blood	7.2–7.6	Anaerobic	10
Beef heart infusion	2% broth	0.05% thioglycollate	7.2–7.6	Air	10
Beef heart infusion	2% broth	0.05% thioglycollate + 10% horse serum	7.2–7.6	Air	10
Beef heart infusion	Broth	0.2% dextrose + 20% horse serum + 0.05% ascorbic acid	7.2–7.6	Air	10
Beef heart infusion	Broth	0.2% dextrose + 20% horse serum + 0.5% ascorbic acid	7.2–7.6	Anaerobic	10
Beef heart infusion	Broth	5% rabbit blood	7.8–8.0	Air	14
Beef heart infusion	2% agar	5% rabbit blood	7.8–8.0	Air (moist chamber)	14
Beef heart infusion	Broth	30% horse serum	7.8–8.0	Air	14*
Beef heart infusion	2% agar	30% horse serum	7.8–8.0	Air (moist chamber)	14
Beef heart infusion	Broth	20% rabbit serum	7.8–8.0	Air	14
Beef heart infusion	1.5% agar	25% rabbit serum	7.8–8.0	Air (moist chamber)	14*
Löwenstein-Jensen		None		Air-sealed caps	21
Löwenstein-Jensen		0.05% thioglycollate		Air-sealed caps	21
Sabouraud's*		2% dextrose			10

* At least 2 or more blind passages were made in this medium.

All media were inoculated with 10% suspensions of mouse brain which were known to contain the agent since they subsequently killed normal mice when injected intracerebrally.

In every case the organisms grown in the tissue cultures were not ordinary bacterial contaminants, since they failed to multiply on blood agar plates. In summary, the piliformis agent grew abundantly in embryonic mouse tissues cultures but lost its virulence for mice after a few such serial passages.

Effect of Storage on the Agent of Tyzzer's Disease

The infectivity of mouse brain or liver tissue, either in the form of 10 per cent suspensions or as blocks of organs, was destroyed by storage in the frozen state for longer than 2 weeks. However, the agent

was regularly recoverable from infected tissues stored at −20° or −70°C. for only a few days.

Discussion

Overcrowding undoubtedly provided one of the important factors in the explosive epizootics of liver disease which occurred among our mice. On each occasion of an outbreak, it was found that the mice, after receipt from the breeder, had been kept for some time under unsatisfactory conditions. The disease returned to a sporadic state when animals in subsequent shipments were provided with adequate quarters. The marginal war diet given mice may have contributed to their susceptibility, but it was probably not a factor in the sharp outbreaks since essentially the same foodstuffs were fed by both the breeder and us. It would appear that the disease was enzootic in the British stock mice and that it flared up when conditions were adverse, a common epidemiological observation. Some evidence for its frequent presence was the finding on a number of occasions of structures which appeared typical of the piliformis organism in stained smears of spleens of apparently normal mice of the same stock. Tyzzer likewise regarded the disease as one which occurred enzootically.

The part the piliformis organism plays in the etiology of mouse liver disease has not been clearly established. In our experience, as in Tyzzer's, this organism was always present in material infectious for mice. Indeed, the incubation period of the experimental disease was usually inversely proportional to the number of organisms seen in infected mouse tissue employed as inoculum. However, in certain instances obvious illness was not induced in mice by inocula rich in the organisms; notably, in those experiments with piliformis grown for several passages on tissue cultures.

These observations may indicate that the piliformis organism is the sole etiological agent of the fatal encephalitis in mice but that it is a fastidious organism which rapidly loses its virulence when grown in tissue culture. On the other hand, they may indicate that piliformis and an associated agent are responsible for the mouse disease and that piliformis grows in tissue culture but the concomitant agent does not. Serological studies, which unfortunately we were unable to complete, should contribute to an understanding of this problem. Van Rooyen [5] has commented on the morphological similarity of *Bacillus piliformis* and *Streptobacillus moniliformis*, but further analogies between these organisms are lacking. Neither is there weighty evidence which suggests that the agent of Tyzzer's disease is a member of the pleuropneumonia group of organisms.[6]

Summary

Tyzzer's disease (epizootic hepatitis) of mice can be transmitted experimentally to mice, rats, hamsters, and rabbits by the intracerebral inoculation of infectious material. The experimental cerebral lesions, like those occurring in the livers of naturally infected mice, consist of focal areas of liquefaction necrosis surrounded by an acute inflammatory infiltration and contain many of the bacteria-like piliformis organisms. The piliformis organism is consistently associated with the natural and experimental disease in animals, and can be grown in tissue culture, but has not yet been cultivated in media devoid of living cells.

The photomicrographs were prepared under the direction of Mr. Roy M. Reeve at the Army Institute of Pathology, Army Medical Museum, Washington 25, D.C., where the negatives are on file.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 106

FIG. 1. Photomicrograph illustrating a typical lesion in the liver of a naturally infected mouse. There is an area of liquefaction necrosis surrounded by a zone of infiltrating cells. Hematoxylin and eosin stain. × 100. (Neg. no. 93334.)

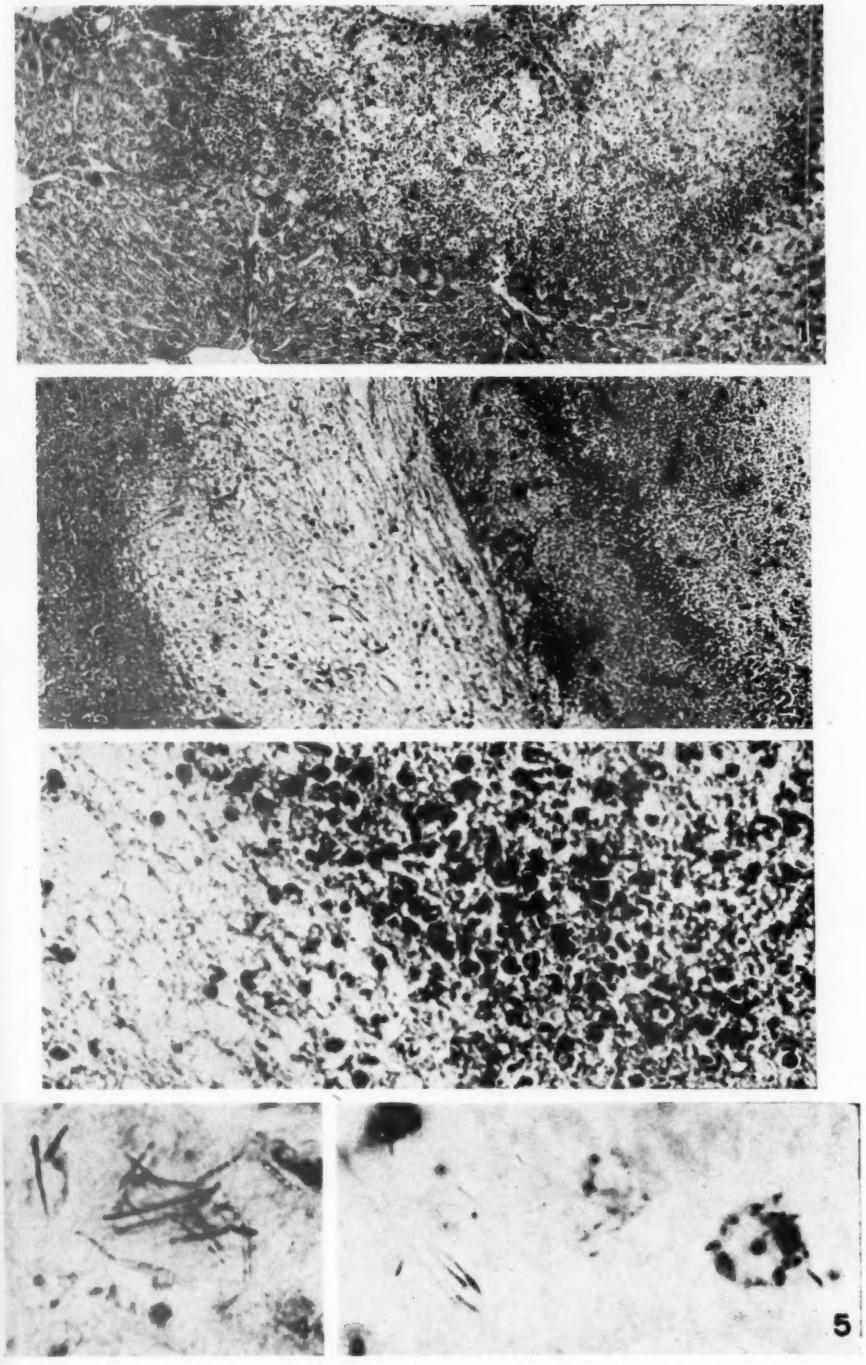
FIG. 2. Photomicrograph illustrating acute necrosis in the brain of a mouse with experimental encephalitis. Hematoxylin and eosin stain. × 100. (Neg. no. 93157.)

FIG. 3. Photomicrograph showing the edge of a zone of necrosis in the brain of a hamster injected intracerebrally. Polymorphonuclear cells are found in the disorganized adjacent area. Hematoxylin and eosin stain. × 600. (Neg. no 93339.)

FIG. 4. Photomicrograph of piliformis organisms found in the hepatic lesion of a mouse with the natural disease. Of note is the characteristic banded appearance of the organisms. Gram's stain (Weigert's method). × 1350. (Neg no. 93155.)

FIG. 5. Photomicrograph of a section of the brain of a mouse dying from experimental encephalitis. Clusters of the organisms are seen in the remnants of cells and lying loose in the tissue. Gram's stain. × 1360. (Neg. no. 93340.)

Righ



Rights, Jackson, and Smadel Tyzzer's Disease in Mice

THE HISTOPATHOLOGY OF ACUTE MUMPS ORCHITIS *

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Orchitis developing in association with mumps is universally recognized as a prevalent concomitant of this disease.[1] Frequency of occurrence varies with age and with each epidemic. With the exception of encephalomyelitis,[2] it is probably the most serious complication of the mumps syndrome. The possibility of residual atrophy with sterility lends particular interest to the malady.[1]

Despite the interest and attention which have been directed to the disease, there is comparatively little knowledge regarding the underlying pathologic lesion or its mode of development. This is not surprising in view of the low mortality rate, the self-limited character of the process, and the infrequency with which surgical therapy has been invoked. Stolz,[3] Reuscher,[4] Hall,[5] and Malassez [6] have described the late lesions appearing in chronic orchitis. Only in the case described by Manca [7] and in the two cases recorded by Smith [8] is there information pertaining to acute mumps orchitis in the human being. Findlay and Clarke [9] observed the testicular lesions in monkeys with experimental mumps.

In the course of a previously reported epidemic of mumps among military personnel,[10] the procedure of orchidotomy as suggested by Wesselhoeft and Vose [11] was carried out by surgeons upon approximately 85 patients with orchitis. The proponents of the method believed that incision through the tunica albuginea testis would serve to relieve intratesticular tension and thus avoid the sequelae of necrosis and atrophy. In carrying out this procedure it was noted that parenchymatous substance immediately bulged through the incised capsule. Accordingly, minute fragments were removed for histologic study.

Seventy-five such fragments, none of which exceeded 0.4 cm. in diameter, were found to be suitable for histologic study. Since this form of therapy was considered to be indicated only early in the course of the disease, none of the material received represented the lesion beyond the fifth day. The majority of the patients were orchidotomized within 48 hours of the onset of symptoms. One additional patient with mumps complicated by bilateral orchitis and femoral thrombophlebitis succumbed to massive pulmonary embolism 11 days after the onset of orchitis. Material from both testes obtained at autopsy in this case

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and the 75 specimens obtained for biopsy serve as the basis for this report.

In most of the fragments studied, a strip of tunica albuginea was included with the parenchymatous tissue. In 18 instances there were portions of extratesticular and extra-epididymal appendages included. With one fragment accidentally, and in the testes from the necropsied case, there were portions of the epididymis available for study. Zenker's fluid was utilized as a fixative for the autopsy material and 10 per cent formalin for the biopsy tissue. Sections were cut in paraffin and stained with hematoxylin and eosin. Connective tissue stains and examination for bacteria and inclusion bodies served no additional purpose.

Histologic Observations

Despite the fact that all but one of the specimens were obtained within 5 days of the onset, there was nevertheless a wide variation in the extent and intensity of involvement. In view of the rapidity with which the disease reached its peak of clinical intensity, this variation appears understandable. The telescoping of range of transition within a brief period would naturally permit a rapid change in the nature of the lesion. Under such circumstances it was impossible to establish a consistent correlation between clinical duration and the character of the lesion. In part, this was due to the variation in intensity of the disease in different patients. Moreover, it seemed evident also that scrotal pain was not an infallible criterion of the onset of orchitis, for in certain instances it resulted from epididymitis or inflammation of extratesticular appendages. Such a condition, masking or mimicking orchitis as it might, precludes the acceptance of pain as an index of either duration or intensity.

On histologic grounds, however, there seemed reasonable evidence of a divisible developmental trend. Consistent microscopic features appeared to justify the assumption that the acute lesion in the testis was susceptible to subdivision into four fairly distinctive phases.

Testis

In 6 specimens of testis it was impossible to detect any abnormality whatever (Fig. 1). There was normal structural arrangement and no evidence of either inflammation or edema. In each of these patients symptoms had been present for 24 hours or less, and it is presumed that they may have arisen either from involvement of the extratesticular appendages or from intratesticular foci not included in the sections.

The earliest abnormality recognized in 31 preparations was interstitial edema. This was manifested in the tunica albuginea by separa-

tion of connective tissue fibers and in the parenchyma by broadened interstices, fraying and floating of connective tissue elements, precipitated fluid, and separation of seminiferous tubules (Fig. 2).

Germinal epithelium exhibited minor degenerative changes such as cytoplasmic swelling, increased depth of nuclear staining, and desquamation of surface epithelium into the lumen. Spermatogenesis, however, appeared to proceed in unimpeded fashion.

The next (second) phase, exemplified by 10 specimens, was characterized by definite, but relatively slight, reactive change. There was a considerable degree of vascular dilatation and engorgement, particularly in the deep portion of the tunica albuginea and in the loose fibrillar zone intermediate between capsule and parenchyma. Small numbers of lymphocytes were clustered about capsular blood vessels. Interstitial edema persisted in the parenchymatous region and, in addition, arterioles showed mural thickening. This was in part the result of swelling of smooth muscle components and in part due to proliferation as evidenced by increased numbers of smooth muscle nuclei. Here, as in the capsule, there was a scant, loose, perivascular aggregation of lymphocytes (Fig. 3). Germinal epithelium showed little additional degeneration, although many of the tubular lumina contained precipitated fluid in addition to desquamated elements.

Fifteen specimens showed progression to a more advanced (third) stage. Perivascular lymphocytic collars in the capsule were wider and more thickly populated and there was now a sprinkled infiltration of the edematous capsule as a whole by similar cells. As before, in the deep portion of the capsule and in immediate subcapsular stroma, the reaction was more pronounced. Not only were lymphocytes more numerous here, but there was also an irregular hemorrhagic extravasation. Many arterioles showed mural thickening (a single instance of arteritis with thrombosis was encountered), but as a rule evidence of unquestionable intrinsic vascular damage was lacking. There was no arterial necrosis. Polymorphonuclear leukocytes, though present, were sparse, but there was a rather pronounced membrane-like deposit of fibrin particularly evident in the immediate subcapsular region.

Within the parenchyma itself the lesion was distinctly spotty (Fig. 6). Many areas retained the appearance described in the earlier stages. Here and there the perivascular lymphocytic reaction was more intense and spread in an irregular manner within the intertubular tissues. Clusters of Leydig cells were not primarily affected but were often partially obscured by the advancing exudate. In some areas lymphocytes were so numerous that they completely filled, in a closely packed manner, the space intervening between tubules (Figs. 4 and 5).

Where the exudate was less pronounced, perivascular aggregations of lymphocytes attained considerable depth. There were an associated scanty fibrin deposit and comparatively few polymorphonuclear leukocytes, the latter exhibiting no focal concentration. There were also interstitial hemorrhages which, though usually small, were in some instances widespread and coalescent. In such circumstances encompassed tubules were sharply outlined by the brightly staining mass of erythrocytes. Neither thrombosis nor necrosis appeared in the interstitial tissues.

During this phase, for the first time, inflammatory elements were noted within the seminiferous tubules. This reaction was also focal and appeared but rarely in areas free from interstitial exudate. Its composition differed from that of the interstitial process in that the exudate was composed preponderantly of polymorphonuclear leukocytes. Among these cells were a few phagocytic macrophages. The germinal epithelium exhibited progressive degeneration with pyknosis and cytoplasmic fragmentation. Ultimately all of the epithelium in an affected tubule was dislodged from its mural attachment, spermatogenesis ceased, and an agglomeration of fragmented cells, débris, polymorphonuclear leukocytes, and phagocytes formed in the lumen. Only Sertoli cells remained attached to the lamina propria of the tubule. These cells, though frayed, did not at this time appear intrinsically damaged. These supporting elements seemed to coalesce and form a narrow tessellated syncytium lining the tubule. At the same time the lamina propria showed a segmental arcuate thickening which eventually involved the entire circumference. This apparently was initiated in those portions adjacent to interstitial blood vessels, the adventitia of which contained reactive exudates. The thickening seemed to result partially from fibroblastic proliferation, partly from infiltration by inflammatory exudate, and partly by apparent fibrosis of residual Sertoli elements (Fig. 5). The intramural exudate, comprised initially of lymphocytes, became polymorphonuclear in character as the intraluminal lesion progressed.

It could not be determined with certainty whether or not this change occurred along the entire longitudinal extent of a tubule. From the appearance of those sectioned sagittally and the larger sections available from the necropsy, it appeared that this was indeed the case. In other words, though focal and spotty with regard to the testis as a whole, individual tubules were involved throughout their entire extent.

The most advanced acute lesion (fourth stage) in this study was observed in 13 specimens. Although focal distribution was maintained,

there was evidence of a tendency for the involvement to become diffuse with few of the parenchymatous structures remaining uninvolved (Fig. 7). Capsular changes had not progressed beyond those previously described. The interstitial tissue, however, was completely filled with densely packed lymphocytes, among which relatively small numbers of polymorphonuclear leukocytes and macrophages were evident. In some cases a thick intertubular fibrin deposit was noted and in others there were many foci of hemorrhage. The lumina of the tubules now contained no viable germinal epithelium, nor was the Sertoli cell syncytium apparent. Instead, the tubules were distended and plugged by dense masses of polymorphonuclear leukocytes which lay enmeshed within a delicate fibrin network (Fig. 8). Interspersed among these cells were fewer but variable numbers of macrophages, lymphocytes, and fragments of cellular débris. The lamina propria, though thickened and infiltrated by leukocytes, was intact and there was no evidence of destruction of sustentative elements. Despite the degree of damage, it would seem from the nature and extent of the changes that complete atrophy of the testis would be an unusual sequela. That permanent damage may result focally, however, was shown in the later stages observed in the patient who was studied by necropsy.

Material from both testes was examined in the fatal case. The lesions differed in several respects from those noted above, probably as the result of the duration of the process (11 days). They seemed to be more intense than in the surgically procured specimens. Interstitial tissue in all areas (the sections were sagittal and included the entire gonad) was markedly edematous and contained large foci of hemorrhage, a considerable fibrin deposit, and a heavy infiltration of lymphocytes and macrophages. Despite the intensity of this lesion, there were very few polymorphonuclear leukocytes. Many of the phagocytes contained vacuoles and hyperchromatic detritus. The capsular reaction was identical with that described above. Several large groups of tubules showed retention of normal epithelium which was proliferating actively (Fig. 9). The great majority, however, suffered from a variety of changes ranging from desquamation with heavy mural infiltration of lymphocytes and plasma cells to complete cessation of active inflammation with organization of the tubules. This was manifested by increased thickness of the lamina propria and collagenization of both the lamina and the Sertoli remnants attached to the wall. In tubules so affected, permanent atrophy undoubtedly resulted. Between the two extremes cited there were many tubules, the lumina of which were filled with coagulated débris and many lymphocytes and laden

phagocytes. It is interesting that in none of the tubules in this case were polymorphonuclear leukocytes so prominent a feature as in the earlier and more acute lesions described above.

Testicular and Epididymal Appendages

Eighteen examples of testicular and epididymal appendages were studied. All showed some evidence of inflammatory infiltration which was not unlike that seen in the tunica albuginea of the testis. There were edema and congestion of variable degree. Some capillary channels were widely dilated and pool-like in appearance. There was a variable amount of perivascular lymphocytic aggregation which was spotty in distribution. In one case with a history of scrotal pain intermittent for months preceding the attack of mumps, a large number of eosinophils were found interspersed among the lymphocytes. This probably represented a mumps-induced exacerbation of a nonspecific epididymitis. It was the only instance in which granulocytes were found in the lesion of the appendage. Surface epithelial elements and those lining the pinched off downpouchings were wholly intact.

Epididymis

Sections from three epididymides were available (two of these were obtained at autopsy). The appearance of the connective tissue was identical with that described in the testicular capsule and the appendages. In the main there was a focal lymphocytic exudate associated with relatively little fibrin deposit and a rather marked vascular engorgement. The infiltration about the ducts, however, was intense, consisting of closely packed lymphocytes clustered about the tubules and often completely filling the interductal spaces (Fig. 10). The epithelial cells lining the ducts were intact and apparently unaffected. The lumina were usually unremarkable, but in some instances contained masses of desquamated epithelium and débris and even a few small clumps of polymorphonuclear leukocytes. It is believed that these represented products of the orchitic process which had been swept up into the epididymis.

Discussion

Both Wolbach (in Smith [8]) and Manca [7] described lesions which, with but little variation, resembled the process as it was manifested in the present series of cases. Wolbach noted the focal character of the exudate in the tunica albuginea, the edema, small hemorrhages, and perivascular exudates. He found, however, that the reactive cells were not lymphocytes but "endothelial leukocytes" and polymorphonuclear

leukocytes. This was also the case in the parenchymatous process. Here the contrast between intratubular and extratubular exudates was not as striking as in the acute cases in the present series. He found polymorphonuclear cells predominating in both regions. Manca examined both testes in a case which was autopsied several days after the onset of mumps orchitis. In his material, too, there was close similarity to the observations made in the present series. Lymphocytes appeared in great numbers in the interstitial area but polymorphonuclear leukocytes were increased in the more advanced foci. The fibrin deposit seemed to be greater than was apparent in the current group. The epididymitis was identical in appearance with that described above.

The pathogenesis of the lesion remains somewhat obscure. Inclusion bodies similar to those described by Johnson and Goodpasture [12] were not recognized in Giemsa-stained preparations of several of our specimens. It would seem that whatever the cause there was initially a mild but widely distributed vascular injury manifested by edema, congestion, and perivascular lymphocytic clustering. It is possible, as suggested by Wesselhoeft and Vose,[2,11] that with this as an initiating impetus and the limited expansibility of the tough tunica albuginea as a concomitant, the degenerative and suppurative changes were altogether secondary in nature. In other words, the lesion is presumed to represent a combination of mild inflammatory response to the mumps virus and pressure necrosis inherent upon the architecture of the testis. The specific necrotizing effect upon the germinal epithelium in contradistinction to the stimulating effect upon the supporting structures may be attributed to the more highly specialized development of the former elements. Epithelial cells would, under such conditions, be expected to exhibit less resistance to disturbance of nutrition than would sustentative tissues. Another factor which may in part explain the disproportionately greater amount of intratubular reaction is the failure of drainage from tubules plugged by fibrin and desquamated epithelium.

In the absence of evidence of widespread organization of the damaged testis it would not seem likely that persistent significant clinical stigmata might be anticipated. Certainly, complete atrophy should not be expected in the face of the spotty distribution of the lesion. It is probable that scattered tubules might fail to regenerate and, as noted in the fatal case, become hyalinized. It is not unusual to find islands of hyalinized tubules in otherwise normal testes examined incidentally at necropsy. In view of the collagenization observed at 11 days, it is reasonable to assume that these may represent in some instances the residua of previous attacks of mumps. In the unusual case of orchitis, in

which the disease is fulminating and diffuse, it is probable that complete atrophy and, in the event of bilateral involvement, sterility may result. Unfortunately, save for the rare case subjected to delayed surgical intervention or autopsy, interpretations of atrophy recorded in the literature are based upon conclusions reached by clinical examination. Since relative size and consistency of the gonads are used as criteria, interpretative accuracy must be considered equivocal.

Summary

Testicular and epididymal tissue obtained from 76 cases of acute mumps orchitis (75 for biopsy and one at autopsy) have served as the basis for this study. In all cases testicular tissue was available. In 18 cases there was material from the appendages and in 2 (3 specimens) from the epididymis. Material was obtained during the first 5 days of symptoms in all but one case (11 days).

Considerable variation was evident in the character and extent of the lesions in the testis but it seemed that a developmental trend could be detected. From early edema and a scant perivascular lymphocytic exudate the process progressed to a diffuse lymphocytic infiltration of the interstitial tissue with focal hemorrhage and pronounced destruction of germinal epithelium, with plugging of the tubules by epithelial débris, fibrin, and polymorphonuclear leukocytes. The intratubular lesion remained focal in most instances but in a few cases every tubule in a given section was involved. Evidence of collagenization was elicited only in the one late case.

Inflammation of the testicular appendages and the epididymis remained confined to the connective tissue elements and, with a single exception, was wholly lymphocytic in character. Epithelial elements were unaffected in these structures.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 107

FIG. 1. Testicular parenchyma, as observed in 6 cases with scrotal pain and mumps. No abnormality is detected. Tubules show active spermatogenesis and are closely approximated with little intervening stroma. × 200.

FIG. 2. The earliest perceptible change in mumps orchitis. There is pronounced interstitial edema with separation of tubules and little or no cellular exudate. Mild degenerative changes have occurred in the germinal epithelium. × 200.

FIG. 3. A second phase of the lesion, showing in addition to interstitial edema a sprinkled infiltration of lymphocytes having predilection for a perivascular distribution. There is some increase in intratubular degenerative change. × 200.

FIG. 4. The interstitial exudate has increased in intensity. It is almost wholly lymphocytic in character. Spermatogenesis has ceased and a scant intratubular exudate of polymorphonuclear leukocytes and macrophages has appeared. × 200.

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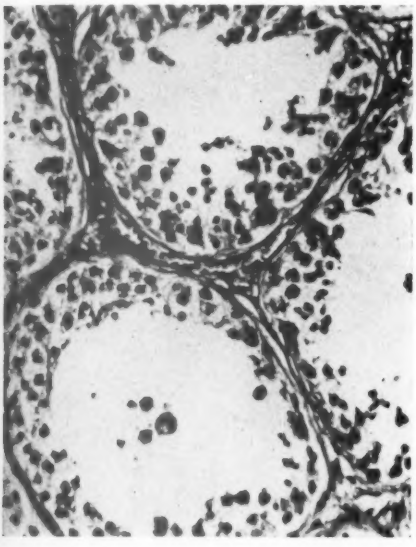

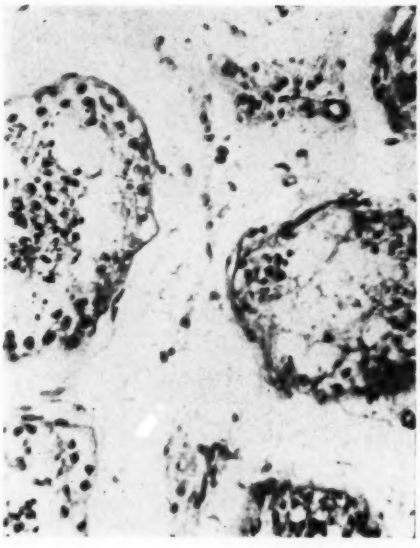

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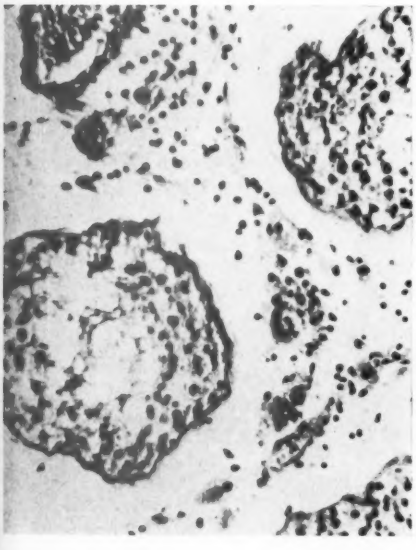

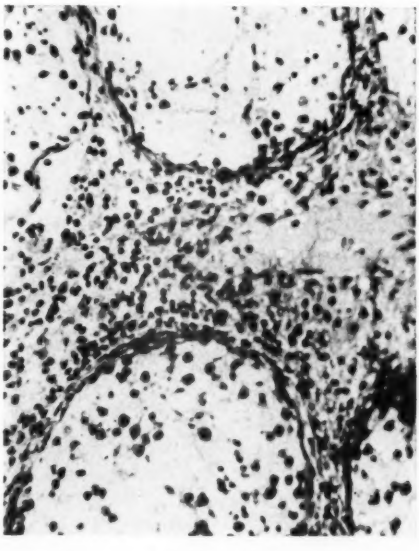

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Gall

Histopathology of Acute Mumps Orchitis

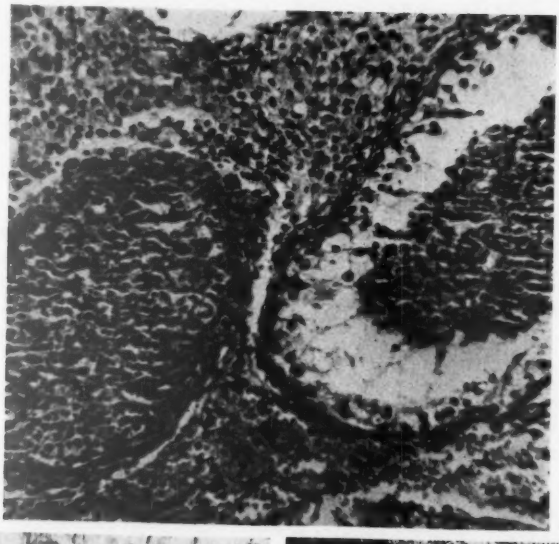
PLATE 108

FIG. 5. In addition to a lymphocytic exudate and hemorrhage in the interstitial tissue, there is now a rather dense polymorphonuclear exudate in the tubules. The lamina propria of the tubule appears thickened and Sertoli cell remnants have fused to form a frayed syncytium. × 200.

FIG. 6. The parenchymatous reaction is shown to be spotty. × 40.

FIG. 7. The process now appears more diffuse and there is suppuration within many tubules. × 40.

FIG. 8. Seminiferous tubules filled completely with a polymorphonuclear exudate. The sole residue of the pre-existing tubule is the moderately thickened lamina propria. Interstitial stroma shows edema, hemorrhage, and lymphocytic infiltration. × 200.



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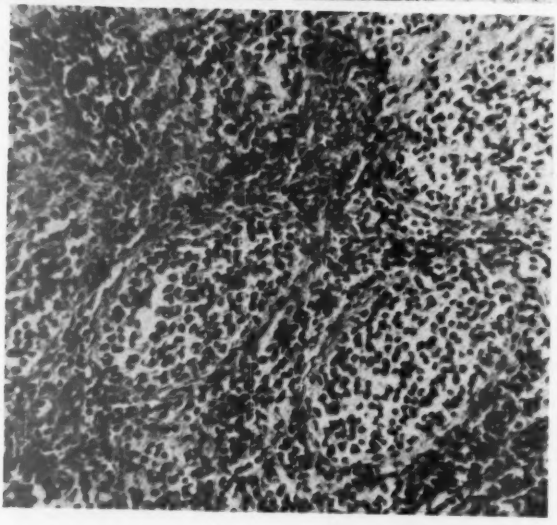

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Histopathology of Acute Mumps Orchitis

PLATE 109

FIG. 9. An area from a testis 11 days after the onset of mumps orchitis. An actively functioning seminiferous tubule contrasts with the surrounding intense inflammatory reaction. Adjacent tubules are the seat of severe degenerative and inflammatory changes. × 200.

FIG. 10. Epididymis in mumps orchitis. There is interstitial edema and a rather pronounced lymphocytic infiltrate. Tubular epithelium is preserved but the lumina contain an exudate presumably swept up from the severe orchitic process. × 200.

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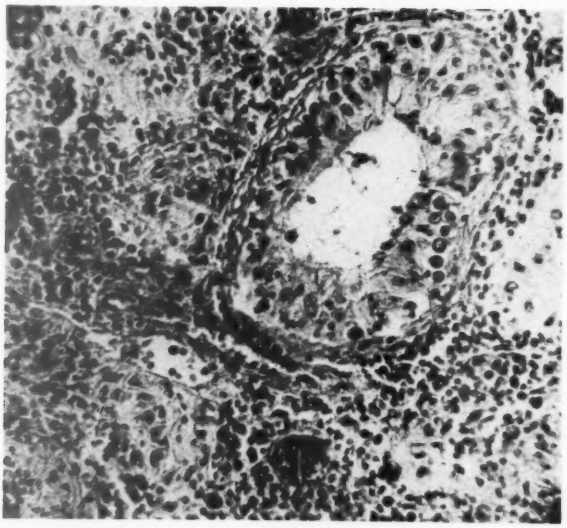

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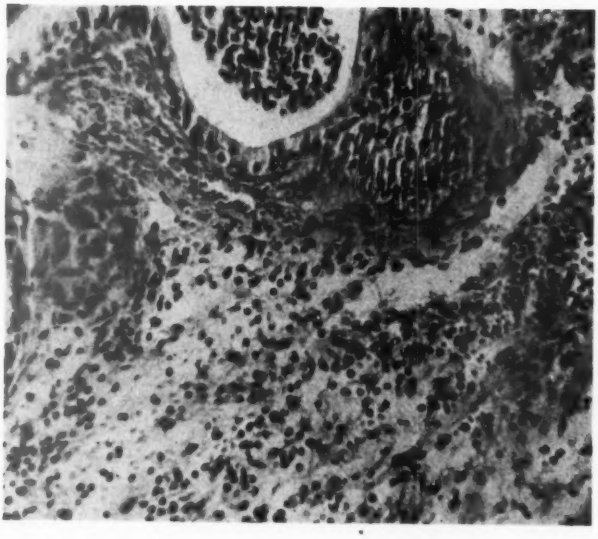

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Histopathology of Acute Mumps Orchitis

LYMPHOGRANULOMA VENEREUM

A Histologic Study of the Primary Lesion, Bubonulus, and Lymph Nodes in Cases Proved by Isolation of the Virus *

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The histologic picture of lymphogranuloma venereum has received little attention since few pathologists have had an opportunity to examine proved lesions of this disease. The diagnosis of this infection is often difficult. Clinical criteria are uncertain; skin and complement-fixation tests are of limited value since they do not necessarily indicate active infection but remain positive long after the disease has subsided. The isolation and identification of the virus is not a practical diagnostic procedure since it is technically difficult. Only recently has it been shown that the complement-fixation reaction can be of value in the diagnosis of active lymphogranuloma venereum.[1]

An extensive investigation of the clinical and laboratory aspects of lymphogranuloma venereum has been completed in this clinic [2] and a number of lesions were excised for histologic study. The majority of the lesions described in this report were proved to be lymphogranuloma venereum by the isolation and identification of the virus. We have not found in the literature any similar histologic studies based on proved material.

Literature

Since several complete reviews on lymphogranuloma venereum have appeared in the recent literature, only a brief summary of the histologic findings will be given here. Textbooks of pathology [3-7] and monographs [8-11] describe the lesions in lymph nodes as follows: The early lesion consists of small masses of epithelioid cells with multinucleated giant cells scattered throughout the gland. These are similar to those seen in tuberculosis or syphilis. The center of the lesion becomes necrotic and contains polymorphonuclear leukocytes, but no true caseation or calcification is evident. Stellate-shaped abscesses are formed and are surrounded by a narrow layer of epithelioid cells which are arranged in palisade formation. Small intracytoplasmic chromatophil bodies of varying shapes are found within large mononuclear cells in the débris of the abscess. These are called "Gamna bodies" and

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were thought to be inclusions associated with the lymphogranuloma virus. The lymph node shows hyperplasia and diffuse inflammatory cellular infiltration. This consists of plasma cells, neutrophilic and eosinophilic polymorphonuclear leukocytes, and basophils, in addition to large mononuclear cells, multinucleated giant cells, and lymphocytes. Fibroblasts proliferate from the thickened capsule into the lymph node. The blood vessels are dilated and may show periarteritis or obliterating endarteritis.

Pund, Greenblatt, and Huie [12] stressed the marked "peritubular" infiltration with plasma cells and lymphocytes, and the dilated lymphatics. Kornblith [13] emphasized that the general architecture of the node is preserved and that the secondary follicles are prominent. The reticulum is intact except in the necrotic center of the lesions. Necrosis is prominent in early lesions while later stages show more fibrosis. After 5 to 6 months the characteristic lesions tend to disappear and only small foci of epithelioid cells surrounded by plasma cells remain in the cortex of the node.

The histologic picture of the primary lesion of lymphogranuloma venereum is rarely described. Hansmann [14] has studied the primary lesion in two patients. These lesions were extragenital and were located on the back of the hand and in the upper dorsal region, respectively. The inflammatory process involved the corium and subcutaneous tissue with small foci of large mononuclear cells, plasma cells, lymphocytes, and occasional giant cells. One of these foci showed central necrosis with many giant cells and resembled tuberculosis. Necrosis, however, was absent in most of the other lesions which were reminiscent of Boeck's sarcoid. The process in the primary lesions was identical with that in the regional lymph nodes. Hansmann also reviewed the descriptions of the primary lesion by Bory [15] and Phylactos [16] who described small ulcerations on the penis with a marked inflammatory cellular infiltration, consisting chiefly of plasma cells and few polymorphonuclear leukocytes. The same cells surrounded the lymphatics. Many newly formed blood vessels were seen and the walls of some of the smaller vessels were thickened. Stannus [11] also reviewed the literature and described epidermal changes with edema of the malpighian and prickle-cell layer and sometimes ulceration. The underlying connective tissue was infiltrated by plasma cells, lymphocytes, swollen connective tissue cells, and cells with several nuclei. The endothelium of the congested blood vessels was swollen but there was little perivascular infiltration. In more advanced cases, however, the process showed characteristic micro-abscesses resembling those found in lymph

nodes. D'Aunoy and von Haam [8] distinguished a herpetiform and chancroidal type. The latter showed necrosis of the epithelium and connective tissue, and dilated lymphatics filled with large endothelial cells. The herpetiform lesion displayed hyperplasia of the granular layer of the epidermis and infiltration of the rete malpighii by round cells and polymorphonuclear leukocytes. An excellent illustration of the primary lesion was shown by Ash and Spitz [3] who described a nonspecific granuloma occasionally containing many eosinophils.

We were unable to find a histologic description of the bubonulus of lymphogranuloma venereum. The lesion is a circumscribed swelling, several centimeters in diameter, which occasionally is found in the prepuce or skin of the penis. It was first described by Brandt.[17]

Method of Study

This report is part of an investigation of the clinical and laboratory aspects of lymphogranuloma venereum in which 28 patients were proved to have this disease by isolation and identification of the lymphogranuloma virus.[18,19] Each of these patients was carefully examined and subjected to a large number of tests. These procedures included skin tests, lymphogranuloma complement-fixation tests, serum protein determinations, formol-gel reactions, biopsies, autoinoculations, cultures, smears, darkfield examinations, and serologic tests for syphilis. The virus was isolated from each of these patients by inoculation of bubo material intracerebrally in mice and into the yolk sac of developing chick embryos. These viruses produced symptoms of meningo-encephalitis in mice and were pathogenic for the developing chick embryo. Smears of the infected mouse brains and yolk sacs revealed elementary bodies of the type found in the psittacosis-lymphogranuloma group of infectious agents. Positive identification of each of these strains as lymphogranuloma virus was made on the basis of their morphologic features, pathogenicity, tissue tropism, sulfonamide susceptibility, and neutralization tests. Smears and cultures of the aspirated material from each patient were negative for the Ducrey bacillus and other bacteria. Darkfield examination of the primary lesions of these patients was negative for *Treponema pallidum*.

The primary lesion of lymphogranuloma venereum was excised from 3 patients subsequently proved to have this disease by isolation and identification of the virus from the associated inguinal bubo. Additional primary lesions were obtained from 4 other patients from whom the virus was not isolated. The clinical and laboratory findings in these 4 patients were consistent with the diagnosis of lymphogranuloma

venereum, and the histologic picture of these four lesions was identical with that seen in patients proved to have the disease. All seven lesions were located on the penile shaft or prepuce.

Inguinal lymph nodes were excised for histologic examination from 2 patients (nos. 171 and 187) and the virus was isolated from these specimens. A third inguinal lymph node was excised from a patient (no. 1) who developed a recurrence of the disease with swelling of the originally infected lymph nodes immediately after a Frei test. The lymphogranuloma virus was isolated from this node. Six months before, the patient had shown clinical and laboratory evidence of acute lymphogranuloma venereum which subsided with treatment. A fourth inguinal lymph node (patient no. 161) also was examined. The virus of lymphogranuloma venereum had been isolated by aspiration of this node 9 months previously. The infection had subsided with treatment and showed no clinical or laboratory signs of activity.

A typical bubonulus located in the prepuce was likewise removed for histologic study and the virus was isolated from this specimen as well as from the associated inguinal lymph node. All of the patients in this series were Negro males.

After excision the tissue from the primary lesion was fixed immediately in Regaud's fluid except for the specimens from 2 unproved cases which were fixed in Zenker's fluid. The inguinal lymph nodes and the bubonulus were sectioned under sterile precautions, and one-half of each specimen was used for virus studies. Slices of the remaining tissue were fixed in Regaud's and Zenker's fluids.

Serial sections were made from all tissues. Material fixed in Regaud's fluid was stained by Giemsa's method recommended by Wolbach and Pinkerton.[20] Zenker-fixed tissue was stained with phloxine and methylene blue. Consecutive sections of the lymph nodes and of the bubonulus were stained with Mallory's aniline blue, Wilder's reticulum, and Weigert's elastic tissue stains. These stains were used only on occasional sections of the primary lesion.

Histologic Observations

The Primary Lesion

The primary lesion was a shallow ulcer in the penile skin or mucosa not more than 2 or 3 mm. deep (Fig. 1). Its fundus consisted of necrotic tissue and was covered by an exudate of fibrin, cellular débris, and some neutrophilic polymorphonuclear leukocytes. Occasional microorganisms, both cocci and bacilli, were found in the superficial layers of the exudate.

An area of dense inflammatory cellular infiltration surrounded the

ulcer. This area was ill defined and merged with the surrounding tissue. Large mononuclear cells predominated but some neutrophilic polymorphonuclear leukocytes also were seen. Plasma cells were numerous toward the periphery of the lesion and a few lymphocytes were present also.

The large mononuclear cells were often aligned along the fundus of the ulcer in palisade arrangement. These cells formed nodules which originated in the adventitia of small blood vessels (Fig. 2). The large mononuclear cells had proliferated and involved not only the surrounding tissue but progressed toward the lumen of the vessel which was gradually compressed and eventually disappeared (Fig. 3). Small solid granulomata were thus formed. The occlusion of the vessel was not associated with significant endothelial proliferation. At this stage the pattern of the vessel wall was visible only with the reticulum stain. Necrosis with polymorphonuclear leukocytes occurred in the centers of these granulomata which then formed small abscesses (Fig. 4).

These granulomata or abscesses might coalesce and this led to the formation of the ulcer. The fundus of the ulcer showed the same architecture and cytologic detail as the granulomata in which abscesses had formed (Fig. 5). Thus the cavity of the ulcer was the necrotic core of the granuloma, the contents of which had been for the most part evacuated. The fundus represented the peripheral zone of large mononuclear cells of the granuloma.

The large mononuclear cells were of the usual type with vesicular nuclei and ample cytoplasm. Multinucleated cells were present but rare. The large mononuclear cells around the areas of necrosis displayed phagocytic activity and contained intracytoplasmic particles. Elementary bodies or microorganisms were not identified.

Some fibroblastic proliferation and newly formed blood vessels were seen at the periphery of the inflammatory infiltration. The blood vessels displayed slight endothelial swelling but there was no appreciable degree of endothelial proliferation. The lymphatics were prominent and dilated. They contained granular eosinophilic material with some large mononuclear cells and rare lymphocytes. The epidermis at the edge of the ulcer showed some polymorphonuclear leukocytic infiltration, edema, and acanthosis but there was no significant repair.

The Bubonulus

The bubonulus consisted of numerous small and large foci of inflammatory cells throughout the deep layers of the skin of the prepuce. Some foci were well defined, while others merged into the surrounding tissue.

The various stages of the lesion were seen in the smaller foci found

around the larger lesions. In the early stages the adventitia of a small blood vessel in a part of its circumference was infiltrated by large mononuclear cells. This infiltration extended from the adventitia toward the intima and separated the layers of the vessel wall. The endothelium over the involved segment was swollen but showed no appreciable proliferation. The cells showed no disruption and no thrombus formation was encountered (Fig. 6). The perivascular lymphatic space was dilated and contained some lymphocytes and large mononuclear cells in addition to granular eosinophilic material. The wall of the lymphatic next to the blood vessel was incorporated in the inflammatory process and showed some swelling of the endothelium.

This infiltration by large mononuclear cells increased and eventually involved the entire circumference of the vessel. The infiltrating cells extended from the periphery of the vessel wall toward the lumen and produced marked separation of the various layers. This cellular infiltration led to the occlusion of the vessel and formation of a solid granuloma as seen in the primary lesion. Small veins were primarily involved while arterial involvement occurred generally at a later stage.

The small solid granulomata consisted of large mononuclear cells with occasional plasma cells and rare lymphocytes at the periphery (Fig. 7). No intracellular inclusions were found at this stage.

Large granulomatous areas were formed by coalescence of smaller lesions and contained several small blood vessels with compressed lumina. These vessels were inconspicuous and might be overlooked, but the reticulum stain revealed their contours (Figs. 8 and 9). This stain also showed that the stroma throughout the granuloma was intact. The elastic fibers, however, were fragmented or had disappeared. No significant connective tissue proliferation occurred at the periphery of the lesion.

After the blood vessels were obliterated, the center of the granuloma underwent necrosis. The reticulum fibers disappeared in the necrotic area and neutrophilic polymorphonuclear leukocytes appeared. At this stage, the necrotic core consisted of polymorphonuclear leukocytes, some fibrin, and cellular débris (Fig. 10). This was derived from the degeneration of large mononuclear cells and polymorphonuclear leukocytes. The large mononuclear cells around the abscess frequently showed palisading, and a few multinucleated giant cells might also be found. The large mononuclear cells often had ingested cellular débris and nuclear fragments but no other intracellular inclusions were seen. At the periphery of the older foci, plasma cells became more numerous and there were occasional eosinophils. Lymphocytes were not present in appreciable numbers. In this manner small abscesses developed which were often branching or stellate.

The tissue between the granulomata showed edema, some fibrin, and scattered plasma cells and large mononuclear cells as well as occasional lymphocytes. Some proliferation of connective tissue and of new blood vessels was noted around the abscess. A large artery was present throughout the lesion and displayed old thrombosis with almost complete organization and extensive recanalization. This vessel was followed through the various levels of the block. It was noted that a large granulomatous lesion reached the adventitia of the artery and extended into the inner layers of the vessel wall where it had produced thrombosis. The endothelium of the blood vessels and lymphatics was otherwise not remarkable. The lymphatics were not surrounded by a cuff of inflammatory cells but were dilated and contained granular eosinophilic material with a few lymphocytes and large mononuclear cells. The epidermis was not affected by the inflammatory process in the underlying tissues.

The Lesion in the Inguinal Lymph Nodes

The general architecture of the inguinal lymph node was preserved. Stellate abscesses were numerous throughout the cortex but were less frequent in the medulla.

The earliest stage of the lesion consisted of small masses of large mononuclear cells. These masses were found chiefly in the cortex beneath the marginal sinus, but they were present also in the medulla. The mononuclear cells arose outside of the germinal centers of the follicles, and could be distinguished from the cells of the latter by their more distinct contours and acidophilic cytoplasm. There was no necrosis and the reticulum was intact. Some of the solid masses of large mononuclear cells enlarged by merging with adjacent lesions. As a result, the marginal and medullary sinuses were obliterated and the lymphoid follicles disappeared (Fig. 11) but the stroma was preserved. The capillaries, however, were compressed by the proliferation of cells around them. Their lumina were reduced in size and were finally obliterated, but the endothelium showed no appreciable proliferation. Also, there was no fibrosis or increased vascularity. These lesions merged with the uninvolved lymphoid tissue.

The compression and obliteration of the capillaries led to ischemic necrosis in the center of the cell masses and eventually resulted in the formation of abscesses (Fig. 12). The reticulum fibers in the necrotic areas became fragmented and disappeared. Neutrophilic polymorphonuclear leukocytes and some strands of fibrin could then be seen. The leukocytes entered the necrotic zone from the capillaries of the adjacent viable tissue.

The abscesses were identical with those seen in the bubonulus. At

first the necrotic center of the lesion was small and was surrounded by a wide layer of mononuclear cells. Later necrosis spread and the encircling mononuclear cells were reduced to a layer of two or three. Small intracytoplasmic inclusions of bizarre shapes frequently were seen in the large mononuclear cells but no elementary bodies were noted. The reticulum fibers at the edge of the necrosis were slightly condensed. Some new capillaries were formed but the connective tissue did not proliferate to any extent.

The uninvolved portions of the lymph node showed large follicles with prominent and active germinal centers. Young lymphoid elements predominated throughout the cortex and medulla, and the lining cells of the sinuses proliferated. The dilated sinuses contained numerous large mononuclear cells in addition to lymphocytes and some neutrophilic polymorphonuclear leukocytes. Plasma cells were found commonly throughout the parenchyma, sinuses, and capsule of the lymph node. Occasional small collections of large mononuclear cells were encountered around the blood vessels in the capsule. These collections were similar to those described in the primary lesion and in the bubonulus. They had not progressed, however, to the formation of true granulomata with obliteration of the blood vessel. The dilated afferent and efferent lymphatic vessels and the tissues at the hilum of the lymph node were not remarkable.

We have examined also an inguinal lymph node from which the virus was isolated during the recurrence of the disease following a Frei test (patient no. 1). This node showed diffuse hyperplasia with active secondary lymphoid follicles. No aggregates of large mononuclear cells or abscesses were seen. There was some increase in small blood vessels and slight fibrosis of the stroma.

Another inguinal lymph node (patient no. 161) was studied which, on aspiration 9 months previously, had yielded the virus of lymphogranuloma venereum. This node showed preservation of the general architecture with a few areas of slight scarring in the cortex and medulla. The small blood vessels and capillaries were increased. The lymphoid follicles were present and often showed active germinal centers. Large mononuclear cells were numerous throughout the node but foci of these cells or abscesses were not found. Some plasma cells and occasional eosinophils were noted. The capsule and the surrounding fat and fibrous tissue showed some fibrosis and slight infiltration by lymphocytes.

Comment

This study of lesions, for which the etiologic factor had been proved, shows that the histologic picture of lymphogranuloma venereum is sufficiently distinct to permit diagnosis. The histologic pattern of

lymphogranuloma venereum is identical in the primary lesion, in the bubonulus, and in the lymph nodes of the disease. The early lesion of lymphogranuloma venereum begins with a focal proliferation of large mononuclear cells which form small aggregates. In the lymph nodes these cells appear first in the cortex, while in the primary lesion and in the bubonulus they arise in the adventitia of small blood vessels near the perivascular lymphatic space. The lumina of the lymphatics show no thrombosis or appreciable endothelial changes. In the lymph nodes the large mononuclear cellular infiltration increases and may obliterate the architecture. In the primary lesion and in the bubonulus progression of the lesion causes obliteration of the perivascular lymphatics. The lesion extends from the outer layers of the vessel toward the lumen. The latter is compressed and finally obliterated without associated thrombosis or endothelial proliferation. In the lymph node a similar compression of the capillaries and small blood vessels occurs. As a result, the lesions in the various locations show small solid granulomatous nodules which consist of large mononuclear cells with occasional multinucleated giant cells. Some plasma cells and rare eosinophils are present at the periphery of the nodules.

The obliteration of the blood vessels leads to necrosis in the granulomata. Neutrophilic polymorphonuclear leukocytes enter the necrotic core from the capillaries at the periphery of the lesion. In this manner stellate abscesses are formed which are characteristic of the fully developed lesions.

Intracytoplasmic inclusions ("Gamna bodies") are seen in the large mononuclear cells only after necrosis has occurred. These inclusions are phagocytized cellular débris. We could not identify in sections the elementary bodies of lymphogranuloma venereum which were seen in spreads of mouse brains and yolk sacs infected with the virus.

The solid granulomata as well as the abscesses may coalesce and cause variations in the general pattern of the lesion. In the primary lesion the process involves the uppermost layers of the skin or mucous membrane. The ulcer represents a coalesced mass of granulomata or abscesses which has broken through the surface and has discharged most of its necrotic contents. Secondary infection is commonly superimposed on the ulcer but in our experience does not materially alter the histologic picture. The formation of multiple fistulae in the lymph nodes of lymphogranuloma venereum probably occurs in the same manner as the formation of the ulcer in the primary lesion, but fistulae did not form in our material.

Plasma cells are numerous at the periphery of a fully developed lesion, but few lymphocytes and only occasional eosinophils are present. A similar diffuse inflammatory cellular infiltration is found between the

granulomata and abscesses. In the acute lesions, fibrosis is present but not marked. The lymph nodes taken from a patient recovering from lymphogranuloma venereum likewise showed only slight scarring. Proliferation of the endothelium plays no appreciable part in the formation of the lesion although some new capillaries are formed at the periphery of the abscesses. Thrombosis was encountered only in a single large artery in the bubonulus and was secondary to the extension of a large granulomatous lesion into the vessel wall.

Except for minor differences, our findings of the lesions in the lymph nodes are essentially in agreement with those recorded in the literature. Significant fibrosis was not encountered even in the lymph node excised 9 months after the acute episode. Giant cells were not numerous in our material. The elementary bodies of lymphogranuloma venereum could not be identified in sections, and intracytoplasmic inclusions in the large mononuclear cells were found only after necrosis had occurred. This finding supports the observations of others that these inclusions are phagocytized débris and are not associated with the lymphogranuloma virus.

Necrosis in the lesions of lymphogranuloma venereum does not appear to be primarily a result of the action of the agent on the tissues. The break-down of tissue results from the obliteration of small blood vessels. These are compressed by the infiltration of large mononuclear cells which progress from the adventitia of the vessel toward the lumen. Endothelial proliferation and thrombosis of blood and lymphatic vessels are lacking in lymphogranuloma venereum.

Serial sections were useful in following the sequence of formation of the lesion. This method was particularly valuable in the study of the bubonulus in which the various stages of the lesion were seen in the deep layers of the prepuce uncomplicated by ulceration and secondary infection. Likewise, the bubonulus did not show unrelated previous lesions which commonly disturb the architecture in inguinal lymph nodes.

Most of the histologic descriptions of the primary lesion in lymphogranuloma venereum which we have found in the literature emphasize only the nonspecific character of the lesion in comparison to syphilis. Hansmann,[14] however, has stressed the similarity of the primary lesion to that in the lymph nodes.

The histologic picture of lymphogranuloma venereum differs from that of other known venereal diseases. In the past the lesions of lymphogranuloma venereum in lymph nodes have sometimes been regarded as similar to those of syphilis and have been called lymphogranulomatous gummata.[11] Our experience shows no resemblance be-

tween the lesions of syphilis and lymphogranuloma venereum. Tuberculosis of the lymph nodes might be considered in the differential diagnosis, particularly since tuberculous infection in the Negro produces a wide variety of pictures. The general pattern of the lesion and the absence of acid-fast bacilli differentiate lymphogranuloma venereum from tuberculosis. The marked endothelial proliferation and vascular changes which are striking in chancroid are lacking in lymphogranuloma venereum. The luxuriant granulation tissue of granuloma inguinale with its characteristic Donovan bodies does not resemble the inflammatory infiltration of lymphogranuloma venereum. In our opinion, the diagnosis of lymphogranuloma venereum can be made with reasonable accuracy by histologic examination alone.

One of the lymph nodes in this series is of particular interest. This case (patient no. 1) is reported in detail elsewhere.[21] When first seen, this patient showed clinical and laboratory evidence of acute lymphogranuloma venereum but the attempt to isolate the virus at that time failed. The disease subsided under treatment and the patient appeared clinically well. A Frei test done 6 months later was followed by recurrence of symptoms and enlargement of the previously enlarged inguinal lymph nodes. One of these nodes was excised and the virus isolated from it. On histologic examination the node showed only hyperplasia and some scarring. It is known that in patients who have had lymphogranuloma venereum, the introduction of the antigen in performing the Frei test may cause a generalized reaction after the disease has apparently ceased to be clinically active.[22] The isolation of the lymphogranuloma virus from a lymph node without morphologic evidence of active infection might be interpreted as lending support to the unproved theory that the immunity in virus infections is due to the persistence of the virus in the cells of the host. It is realized, however, that this observation is open to question since the diagnosis of lymphogranuloma venereum was not proved by isolation of the virus when the patient was first seen in this clinic.

Biopsy is not commonly used for the diagnosis of lymphogranuloma venereum. The primary lesion is evanescent and the removal of lymph nodes is generally regarded as inadvisable because of the possible formation of sinus tracts. A number of lymph nodes of lymphogranuloma venereum have been removed in our out-patient department under local anesthesia without serious discomfort to the patient. The incision healed readily and no sinus tracts formed. The bubonulus in the prepuce was excised in the same manner without untoward results. These experiences lead us to believe that biopsy can be useful in the diagnosis of selected cases of lymphogranuloma venereum since more information

can be obtained from the histologic study than from any other single diagnostic procedure.

Conclusions

The histologic character of lymphogranuloma venereum was studied in twelve specimens. Eight of these were taken from patients in whom the diagnosis of lymphogranuloma venereum was proved by the isolation of the virus. The remaining four patients were considered to have the disease on the basis of their clinical and laboratory findings. These specimens consisted of seven primary lesions, one bubonulus, and four inguinal lymph nodes.

The histologic picture of lymphogranuloma venereum is distinct and is the same in these lesions. Granulomata composed of large mononuclear cells form around small blood vessels which are compressed and obliterated. Thrombosis and endothelial proliferation play no part in the obliteration of the blood vessels which leads to necrosis in the center of the granulomata. Polymorphonuclear leukocytes enter the necrotic core of the granulomata and abscesses are formed. Little fibrosis is seen in either the acute or healed lesions. One of the lymph nodes was removed during recurrence of the disease following a Frei test. This node showed none of the changes of active lymphogranuloma venereum.

The histologic picture of lymphogranuloma venereum is sufficiently distinct to permit a reasonably accurate diagnosis and to differentiate this disease from other venereal infections. For this reason, biopsy appears useful as a diagnostic procedure in selected cases of lymphogranuloma venereum.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

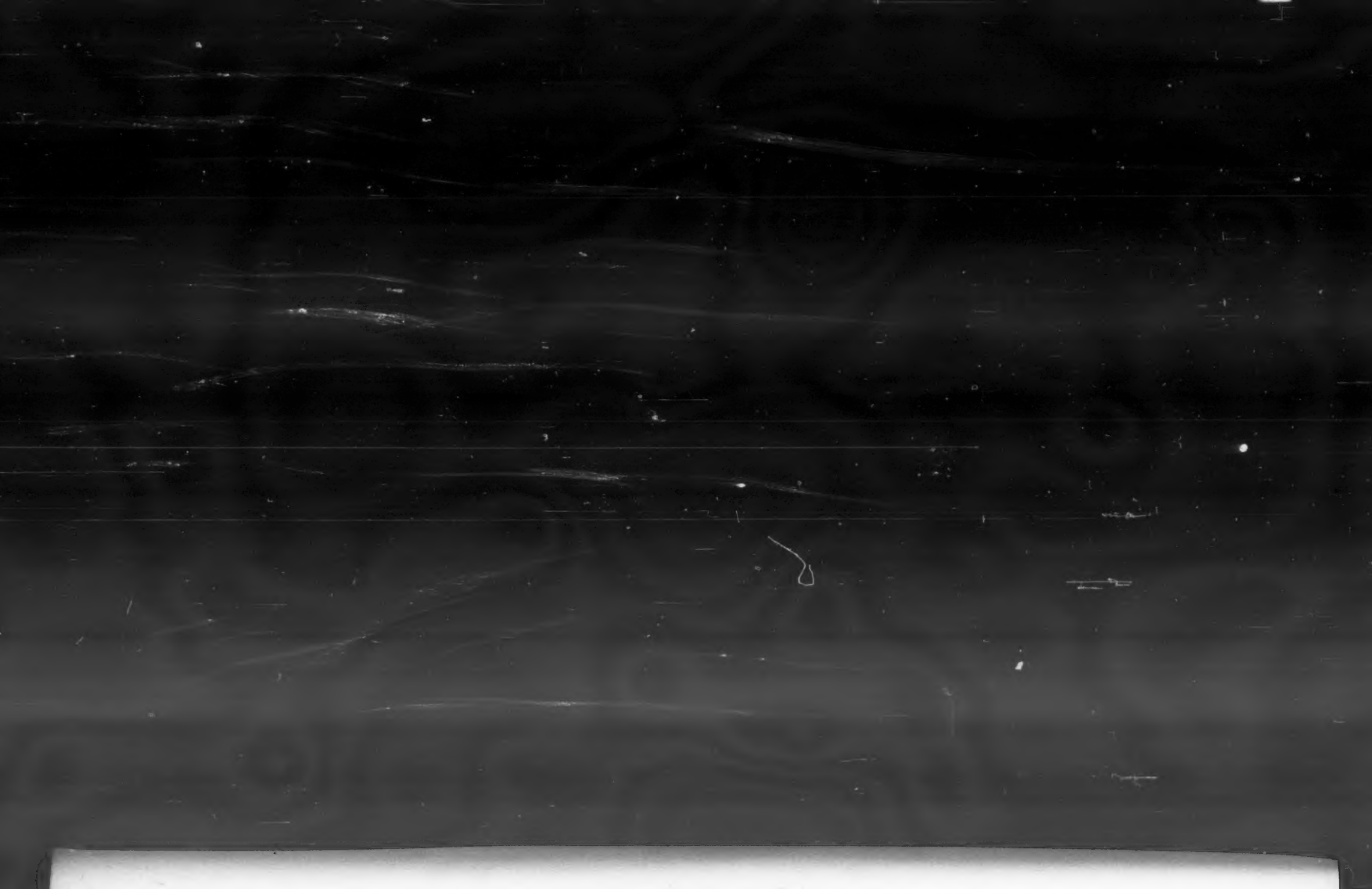
PLATE 110

FIG. 1. Primary lesion. The ulcer is surrounded by an inflammatory cellular infiltration. Giemsa's stain. × 60.

FIG. 2. Primary lesion. A small blood vessel shows beginning infiltration of its walls by large mononuclear cells. The lumen is patent and the endothelium shows no significant proliferation. The single mitotic figure of the endothelium is unusual. Giemsa's stain. × 710.

FIG. 3. Primary lesion. The infiltration of large mononuclear cells in the wall of the vessel has progressed to the formation of a solid nodule. The lumen of the vessel is completely obliterated by compression but the outline of the vessel is still visible. There is no endothelial proliferation. Giemsa's stain. × 500.

FIG. 4. Primary lesion. The early stage of a typical abscess showing neutrophilic polymorphonuclear leukocytes in the necrotic center surrounded by large mononuclear cells. To the right a multinucleated cell is seen. Giemsa's stain. × 420.



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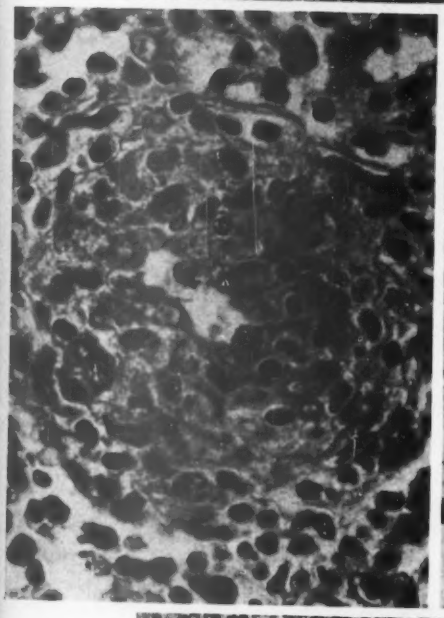

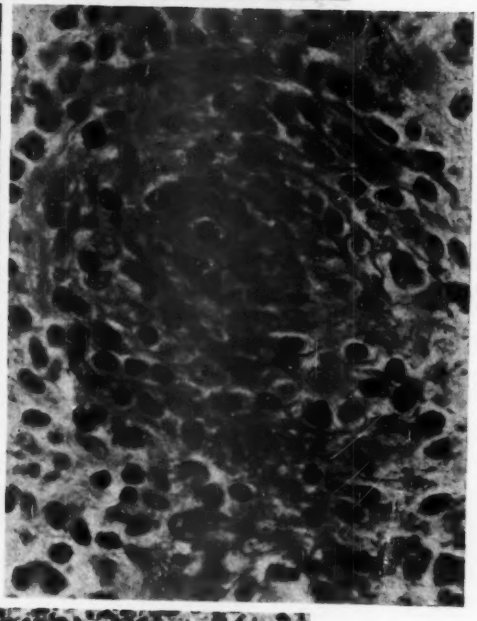

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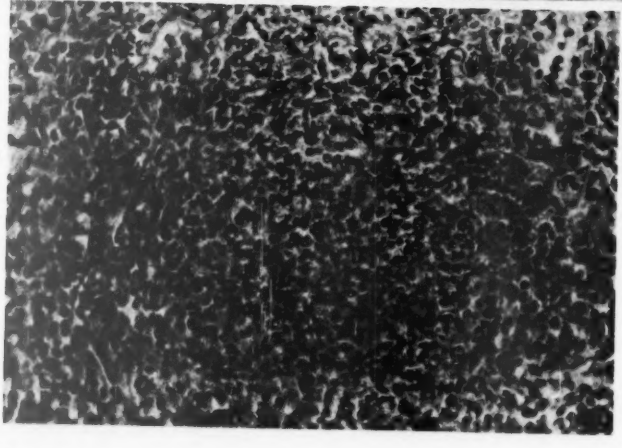

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Sheldon and Heyman

Lymphogranuloma Venereum

PLATE III

FIG. 5. Primary lesion. The fundus of the ulcer is infiltrated chiefly with large mononuclear cells and is covered with exudate. Below and in the center is a small abscess. Giemsa's stain. × 170.

FIG. 6. Bubonulus. The lymphatic space around a small vein is dilated and contains large mononuclear cells. These have infiltrated the wall of the vessel. Phloxine and methylene blue stain. × 345.

FIG. 7. Bubonulus. A large solid granuloma with early necrosis. This lesion has formed by the coalescence of several smaller granulomata. Phloxine and methylene blue stain. × 150.

FIG. 8. Bubonulus. This lesion consists of multiple solid granulomata which have coalesced. Phloxine and methylene blue stain. × 140.

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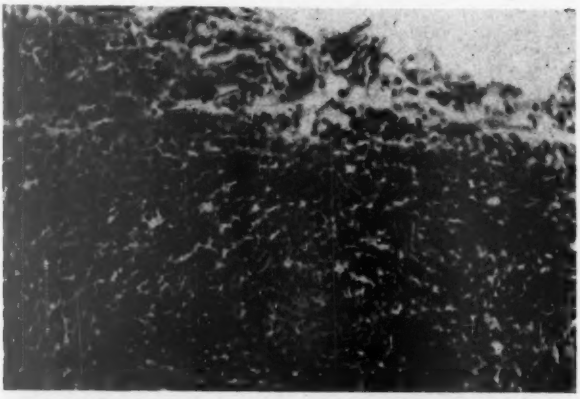

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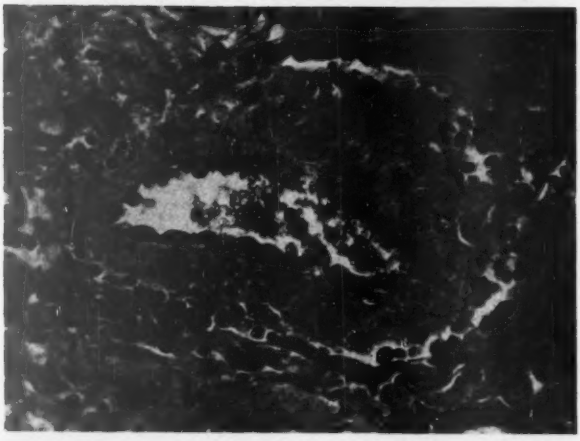

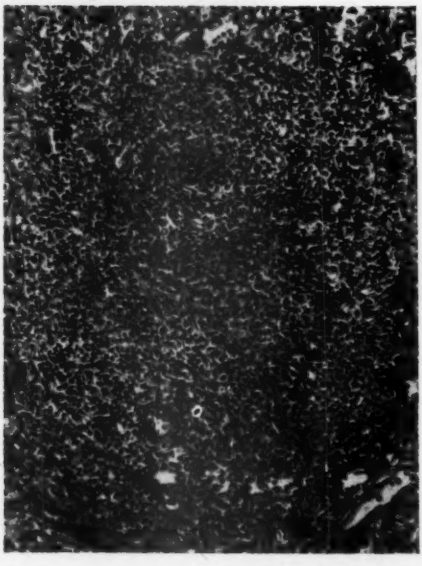

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PLATE 112

FIG. 9. Bubonulus. This is the same field as is shown in Figure 8, but this preparation shows the reticulum and the contours of blood vessels, many of which are masked by the cellular infiltration in Figure 8. Wilder's reticulum stain. × 140.

FIG. 10. Bubonulus. The necrotic core of an abscess with polymorphonuclear leukocytes surrounded by large mononuclear cells. The cavity of the abscess appears branching or stellate. Phloxine and methylene blue stain. × 280.

FIG. 11. Inguinal lymph node. The early stage of the lesion with proliferation of large mononuclear cells and obliteration of the normal architecture. Phloxine and methylene blue stain. × 110.

FIG. 12. Inguinal lymph node, showing a fully developed abscess. Phloxine and methylene blue stain. × 50.

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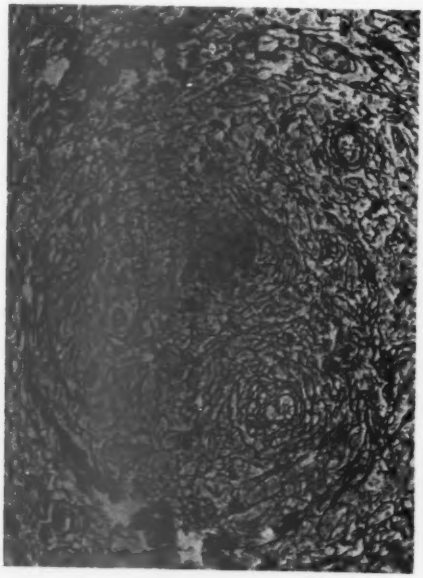

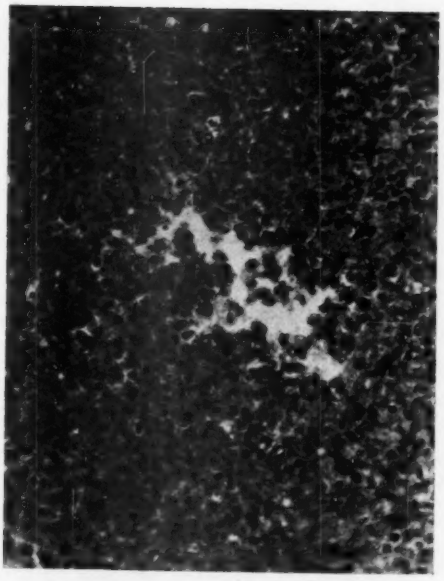

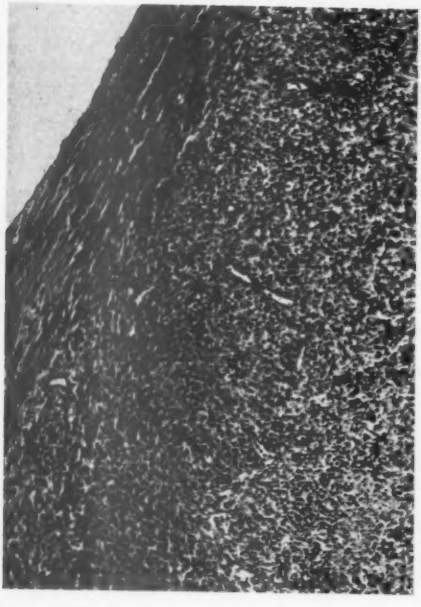

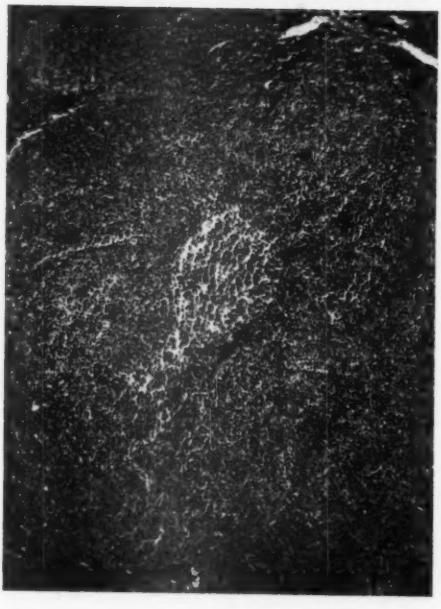

Sheldon and Heyman

Lymphogranuloma Venereum

MUCORMYCOSIS OF THE CENTRAL NERVOUS SYSTEM ASSOCIATED WITH HEMOCHROMATOSIS

Report of a Case *

Philip M. LeCompte, M.D., and William A. Meissner, M.D.
(From the U. S. Veterans' Hospital, West Roxbury, Mass., and The Pathological Laboratory of the New England Deaconess Hospital, Boston, Mass.)

Human infection with fungi of the family Mucoracea is rare. Mucormycosis is not mentioned in the standard American works on pathology, although it is both described and illustrated in one German text.[1] Organisms of this group are referred to as potential pathogens in most works on medical mycology.[2-7] In others they are mentioned as contaminants [8] or "probable pathogens." [9] The literature is reviewed by Gregory, Golden, and Haymaker.[10]

The lungs are the organs most frequently involved, the central nervous system most rarely. The case of Paltauf [11] and the three reported by Gregory, Golden, and Haymaker [10] appear to be the only instances on record of involvement of the central nervous system.

The present case is reported chiefly because of its remarkable resemblance in clinical and pathological details to the three described by Gregory *et al.*[10]

Report of Case

E. A. (R 2104), a white male, 57 years of age, was admitted to the U. S. Veterans' Hospital, West Roxbury, Mass., on September 25, 1944. No adequate history was obtainable. He was semi-comatose, confused, and muttering. The breath had a strong odor of acetone. He did not move his left arm or leg. The right pupil was larger than the left. The right eye was inflamed, swollen, and exuded a purulent discharge. The urine showed 2.5 per cent sugar, a heavy trace of albumin, a few red and white cells, and 3 plus acetone. Blood sugar was 130 mg. per cent; leukocyte count, 12,400, with 84 per cent polymorphonuclear leukocytes, 10 per cent lymphocytes, and 6 per cent monocytes. Hemoglobin was 13 gm. Complement-fixation and precipitation tests for syphilis were negative.

The patient was given 40 units of insulin shortly after admission. The following day he received 100 units with 1000 cc. of 10 per cent glucose. No definite improvement was noted, and he was subsequently given 200 units of insulin with 2000 cc. of 10 per cent glucose. The patient failed to recover consciousness and died on September 27th.

Autopsy (no. 122) was performed by Capt. S. Balkin. The right upper eyelid was found to be markedly swollen and injected. The right pupil was irregular and measured 4 mm. in diameter, while the left one measured 2 mm. About 2000 cc. of clear pale yellow fluid was found in each pleural cavity and 300 to 500 cc. of similar fluid in the peri-

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toneal cavity. The heart weighed 450 gm. and appeared negative. The lungs were congested. The liver weighed 2100 gm., was a dusky reddish brown, and showed numerous small, knob-like elevations of its surface. On the cut surface islets of brown tissue were surrounded by depressed gray tissue. The lower end of the esophagus contained dilated veins. The brain showed congestion of the dura and pia, and there were petechial hemorrhages in the pons, medulla, and cerebellum.

Microscopically, the tissues showed the characteristic findings of hemochromatosis, with deposition of yellow pigment granules giving a positive reaction for iron in the liver, spleen, pancreas, adrenals, kidneys, and heart. There was a well marked cirrhosis of the liver.

The brain showed an acute meningo-encephalitis associated with an abundant large, branching fungus (Figs. 1 to 3). The organism consisted apparently of hyphae branching in a nonseptate or coenocytic manner and measuring about 10 to 14 μ in diameter and up to 200 μ in length. The walls of the hyphae were sharply defined and refractile, while the inner portion stained light blue with hematoxylin. The hyphae showed an extraordinary tendency to invade, infiltrate, and replace the walls of blood vessels and to grow within their lumina (Fig. 3). In many places there was an intense reaction of polymorphonuclear leukocytes associated with the presence of the fungus. In other regions, especially where the hyphae lay within the brain substance, as they occasionally did, the reaction was only slight (Fig. 1).

Unfortunately, no culture was obtained from the tissues at autopsy. Sections of the brain were sent to Dr. John E. Gregory, who stated: [12] "Examination of the sections sent to me by Dr. LeCompte reveals invasion by an organism apparently identical with that present in the three cases previously reported."

Slides and tissue were also sent to Dr. C. W. Emmons, Principal Mycologist of the U. S. Public Health Service, who replied: [13] "I concur in Dr. John E. Gregory's opinion that the fungus seen in these sections is probably Mucor because of the size of the hyphae, the infrequency of cross walls and the manner in which the hyphae branch. In the absence of the culture, I am unable to make any more definite identification of the organism."

Discussion

The association of the fungus infection with hemochromatosis in this instance is presumably accidental as far as the disturbed iron metabolism of that disease is concerned. In other respects, however, the case is remarkably similar to those described by Gregory *et al.*[10] All four cases have exhibited a definite triad, as follows: (1) uncontrolled diabetes;

(2) evidence of orbital infection; (3) meningo-encephalitis associated with the presence of a fungus having the characteristics described above. Another feature common to these cases and mentioned elsewhere in the literature is the extraordinary tendency of the fungus to grow in the walls and lumina of blood vessels. As noted by Gregory *et al.*, much of the necrosis and reaction must be attributed to ischemia produced by the involvement of blood vessels. A similar vascular involvement has been noted in infections with organisms of the Aspergillus group.[6,14]

It seems probable that the fungus gains access to the orbital tissues from the paranasal sinuses and thence invades the brain directly, although such a connection was not definitely demonstrated in the present case. Presumably these cases provide another example of the increased susceptibility of diabetic patients to fungus infections.

Summary

A case of mucormycosis of the central nervous system, apparently the fifth one on record, is described. All four cases reported from this country have had in common the following triad: (1) uncontrolled diabetes, with either coma or mental confusion; (2) evidence of orbital infection; and (3) meningo-encephalitis, with the presence of large, nonseptate, branching hyphae having a peculiar predilection for the walls of blood vessels.

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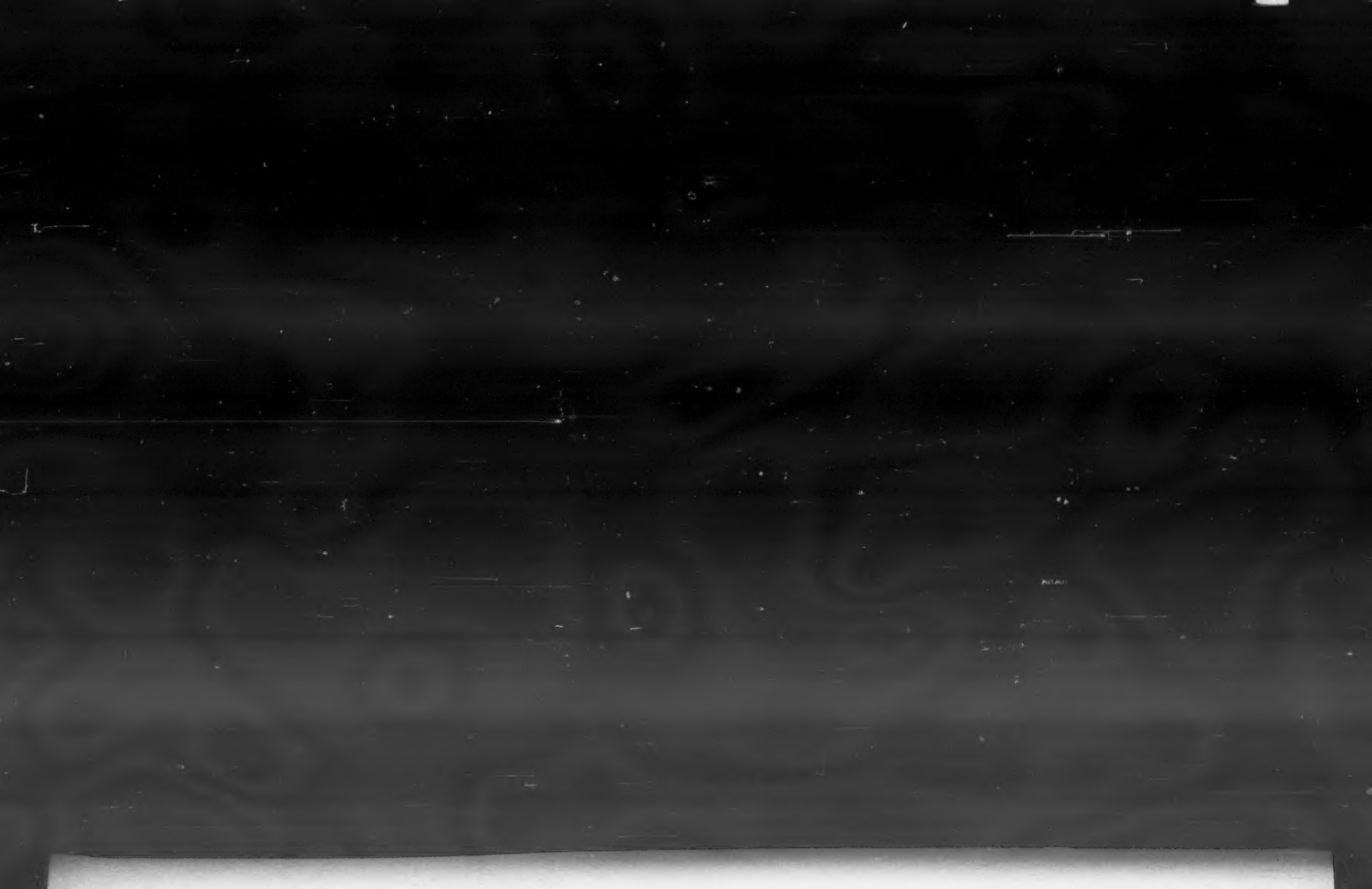
DESCRIPTION OF PLATE

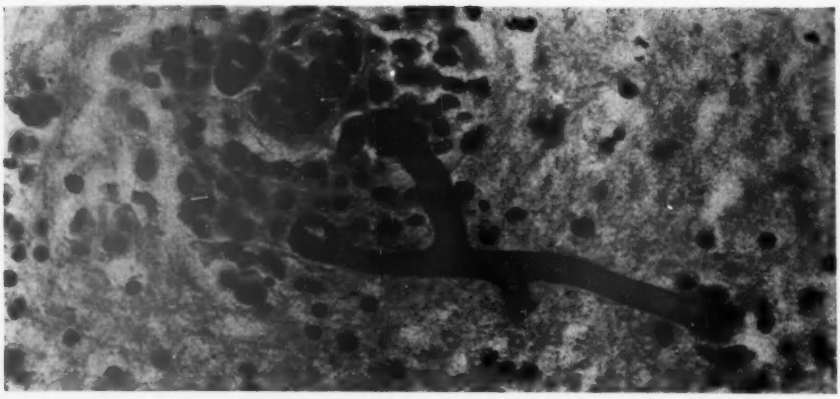
PLATE 113

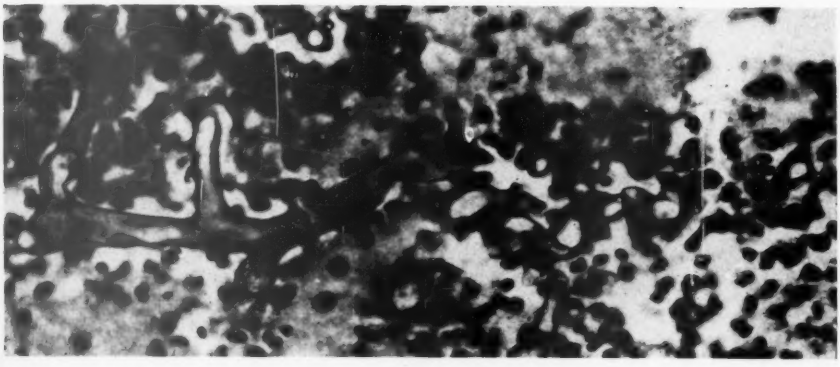
FIG. 1. A nonseptate branching hypha in the brain. In this instance the reaction of the tissue is relatively slight. Hematoxylin and eosin stain. × 500.

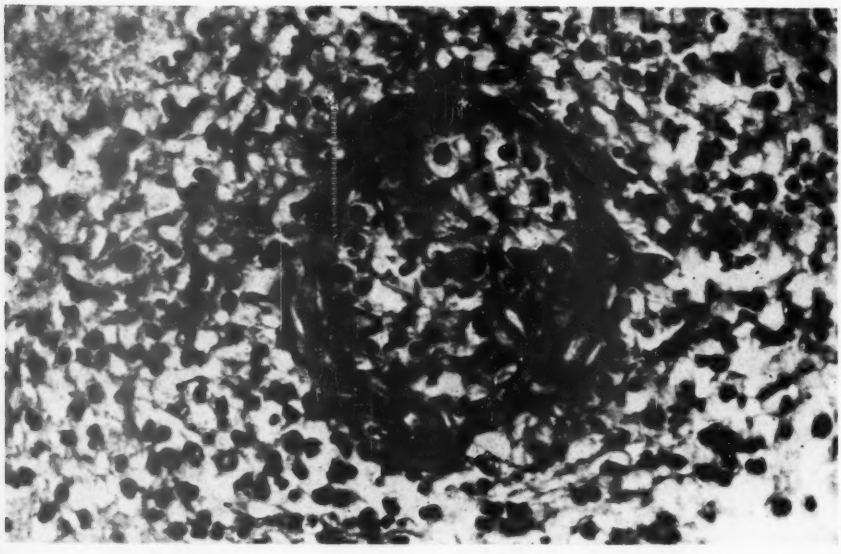
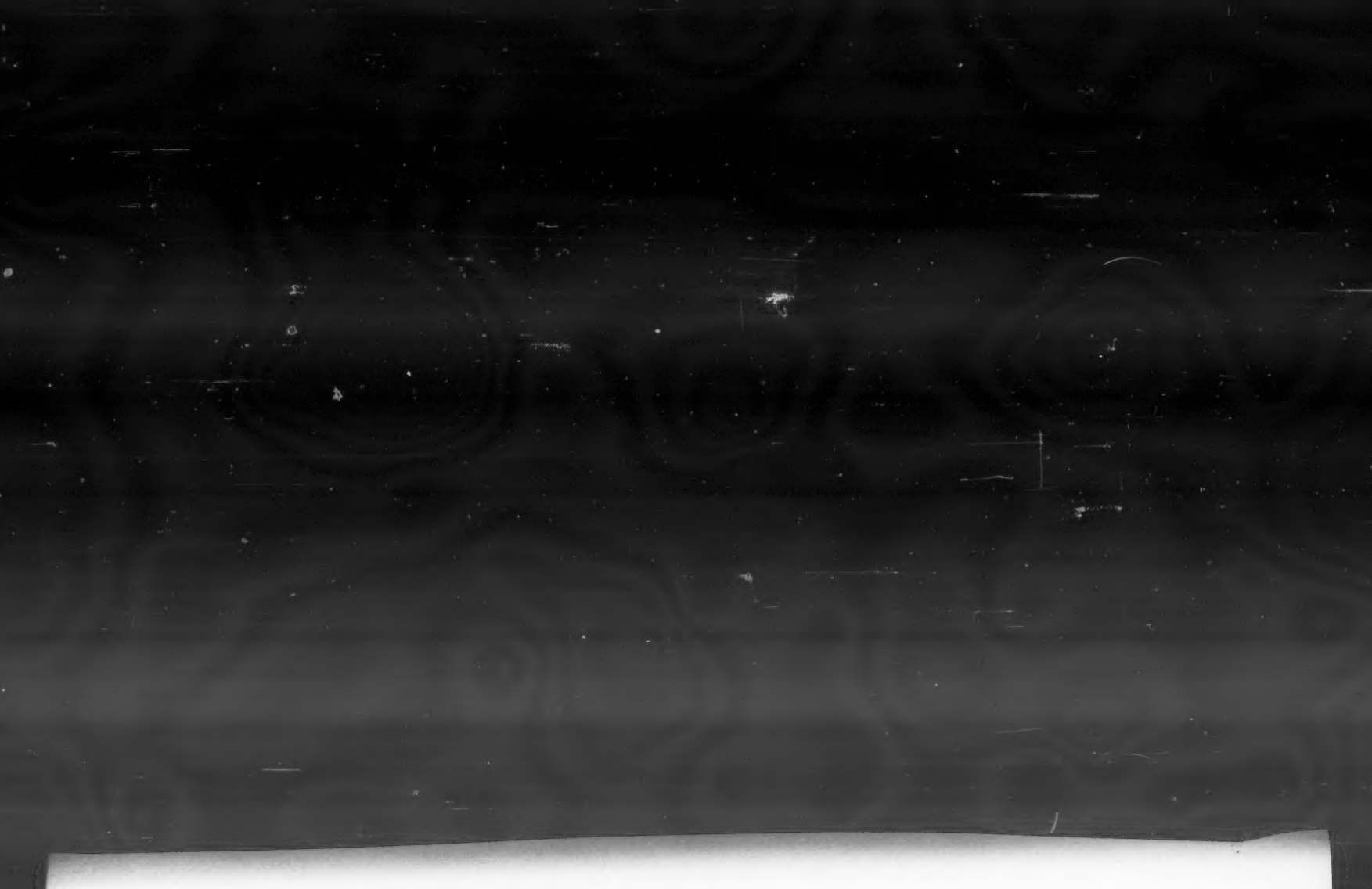
FIG. 2. Hyphae in the brain tissue, accompanied by a marked polymorphonuclear leukocytic reaction. Hematoxylin and eosin stain. × 500.

FIG. 3. Small meningeal vessel in a cerebral sulcus. The fungus has almost replaced the wall of the vessel and is growing in the lumen. Hematoxylin and eosin stain. × 500.









LeCompte and Meissner Mucormycosis with Hemochromatosis